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TITLE OF THESIS..STUDIES...ON...THE..ELECTROPHYSIOLOGY
...OF..RABBIT..SMALL..INTESTINE..
.....

UNIVERSITY.....ALBERTA.....

DEGREE FOR WHICH THESIS WAS PRESENTED....Ph.D.....

YEAR THIS DEGREE GRANTED.....1973.....

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(Signed)..Taher M.-Sharkawy

PERMANENT ADDRESS:

Institute of Physiology...
University of Louvain
LOUVAIN, BELGIUM

DATED..Dec..17.....1973

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THE UNIVERSITY OF ALBERTA

STUDIES ON THE ELECTROPHYSIOLOGY OF RABBIT SMALL INTESTINE

by

© TAHER YOUSSEF MAHMOUD EL-SHARKAWY

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHARMACOLOGY

EDMONTON, ALBERTA

SPRING, 1974

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled STUDIES ON THE ELECTROPHYSIOLOGY OF RABBIT SMALL INTESTINE submitted by TAHER YOUSSEF MAHMOUD EL-SHARKAWY in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

E. E. Daniel
.....
Supervisor

R. B. Stein
.....

George B. Frank
.....

R. Schmidt
.....

.....

.....
External Examiner

Date December 14, 1973

ABSTRACT

The cellular basis of the myogenic control of intestinal motility is the spontaneous periodic oscillations of the membrane potential (MP) of the smooth muscle cells known as the control potentials (CP) or slow waves. They periodically increase membrane excitability so that spiking can be initiated at their depolarizing phases. Previous work suggested that they may be due to oscillations in either the activity of an electrogenic sodium pump or sodium permeability. We further investigated the ionic mechanisms underlying the CP longitudinal muscle strips of rabbit jejunum using intracellular microelectrodes.

(1) The CP of most cells appeared as a depolarization phase, a plateau and a repolarization phase. In a few cells a notch persistently appeared early in the plateau phase. We showed that notching is not an artifact resulting from either contraction or electrotonic interaction with neighbouring cells but may reflect the operation of two sequential processes during the CP; the first causes the initial depolarization and the second leads to the secondary depolarization in notched CPs or to the plateau in un-notched CPs.

(2) K admission to Na-rich tissues hyperpolarized the cells beyond the K-equilibrium potential. The hyperpolarization was prevented by ouabain, continued K withdrawal, cooling and in Li-rich tissues to which K was admitted in the absence of Na. Thus the Na pump in this muscle is electrogenic. Theoretical analysis showed that, under normal conditions, its contribution to the MP is smaller than that predicted from the oscillating pump hypothesis of CP generation.

(3) Sodium-pump inhibition abolished CPs, but the MPs at the time CPs disappeared was more negative than the peak of the CP. Na-pump stimulation by admitting K to Na-rich tissues led to hyperpolarization and the appearance of low amplitude CPs. Cooling reduced CP frequency, prolonged CP duration and intercontrol-potential periods and increased frequency of cells showing notched CPs. The amplitude and rate of initial depolarizations had low Q_{10s} while the duration and rates of onset and offset of secondary depolarizations had high Q_{10s}. Reduction of Na below 20 mM or withdrawal of Ca, but not addition of Verapamil, abolished CPs. Replacement of Cl by less permeant anions hyperpolarized the membranes, decreased CP frequency and abolished the secondary depolarization without seriously affecting the initial depolarization. When Cl was replaced by more permeant anions, a slight depolarization, increase in CP frequency and in frequency of spiking, but no observable effects on CP configuration, occurred. We suggest that the CP results from a Ca-dependent increase in Na permeability (initial depolarization) shortly followed by a transient increase in Cl permeability (secondary depolarization).

(4) Some cells showed slow "intercontrol-potential depolarizations." These depolarizations may trigger the increase in Na permeability in cells that drive the preparations. The sensitivity of the CP frequency to anions suggests that the intercontrol-potential depolarization may be due to a slow increase in Cl permeability.

(5) Adrenaline caused cessation of response activity followed by hyperpolarization but had no effect on CP frequency or configuration or the intercontrol-potential depolarizations. The hyperpolarization may be due to an increase in K permeability and not to stimulation of the electrogenic Na pump.

ACKNOWLEDGMENT

I would like to express my sincere gratitude and indebtedness to my supervisor, Dr. Edwin E. Daniel, who provided generously of his time and effort during the execution of this project. His vast experience and questioning mind enabled him to guide an unsure ship through narrow straits and swifting currents to a safe shore. In moments of despair, during a continuous struggle to resolve a difficult question with a difficult methodology, he gave the encouragement and understanding that made the work possible. His devotion and enthusiasm, day after day, have been the living example for me to follow. If I have learned from his dedication, persistence and enthusiasm, then can I feel I have partially paid my indebtedness to him. I am also grateful to the members of my supervisory committee for their helpful discussions and encouragement.

I also acknowledge the help of Mr. R. Hitkari and Dr. D. Cook for their help with the computer programming, Mr. G. Duchon for the electron microscopic examination of some tissues and Mr. F. Loeffler and Mr. K. Burt for their excellent drafting and photographic work.

My thanks are also due to Mrs. Sue Secker for her skillful typing of this thesis.

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RESULTS AND DISCUSSION

INTRODUCTION

The motor function of the gastrointestinal tract is considered to be a consequence of the myogenic electrical activity of the gastrointestinal smooth muscle cells. The myogenic electrical activity can, in turn, be controlled by extrinsic and intrinsic neural activity as well as by chemical means; e.g., release of gastrointestinal hormones (Daniel and Chapman, 1963; Daniel, 1968, 1969, 1973; Bass, 1968; Holman, 1968; Prosser and Bortoff, 1968; Bortoff, 1972; Baker, 1969). The understanding of the physiology and pharmacology of gastrointestinal motor function requires a complete understanding of the myogenic electrical activity and the ionic events responsible for it occurring at the smooth muscle cell membrane and the basis of its modulation by neural activity and hormonal action. Such information may also prove relevant in promoting our knowledge of the pathophysiology of gastrointestinal motor disorders.

Alvarez, the pioneer of gastrointestinal electrophysiology, commenting on the then current studies on the innervation of the gastrointestinal tract stated that "So many workers, unfortunately, seem to begin without first making that survey of the field which would enable them to see where further work, and especially work with a new technique, is needed." (Alvarez, 1928). Commenting on this statement Holman (1968) wrote "This criticism is just as applicable to many of the papers on the motility of the tract and its innervation published in the 1960s. This is so, despite the development during the last forty years of techniques that enable the physiologist to study the

activity of smooth muscles from new and exciting points of view. Unfortunately, the efforts of many are still directed toward the repetition of studies on the contractions of isolated segments of the small bowel of common laboratory animals, although fundamental questions about the behavior of smooth muscle remain unresolved."

CHAPTER 1

ELECTRICAL ACTIVITY OF SMALL INTESTINAL SMOOTH MUSCLE

A. Anatomical Considerations:

1. The Muscle Coat:

The wall of the small intestine consists of the outermost serosal layer, the muscle coat or the muscularis externa, the submucous layer, the muscularis mucosa and the innermost mucous membrane (see Schofield, 1968; Burnstock, 1970). The anatomy of the intestinal wall and its innervation are schematically depicted in Fig. 1. The muscle coat consists of two distinct smooth muscle layers designated the "outer" or "longitudinal" and the "inner" or "circular" layers. There is considerable species variation in the thickness of each layer.

It was originally thought that the two muscle layers are arranged in the form of open and closed spirals rather than longitudinally and circularly. In an early study on the pig intestine (Carey, 1921) the longitudinal and circular muscle layers were found to be disposed in a left-hand helicoidal arrangement, so that the circular muscle fibers were wound in a close spiral making a complete turn every millimeter or so of intestine and the longitudinal fibers formed an open spiral making a complete turn in about 50 cm. However, in a more recent study on dogs, pigs, cats and man, Elsen and Arey (1966) were unable to confirm Carey's observations. They reported that although some muscle fiber bundles deviated from a strictly longitudinal

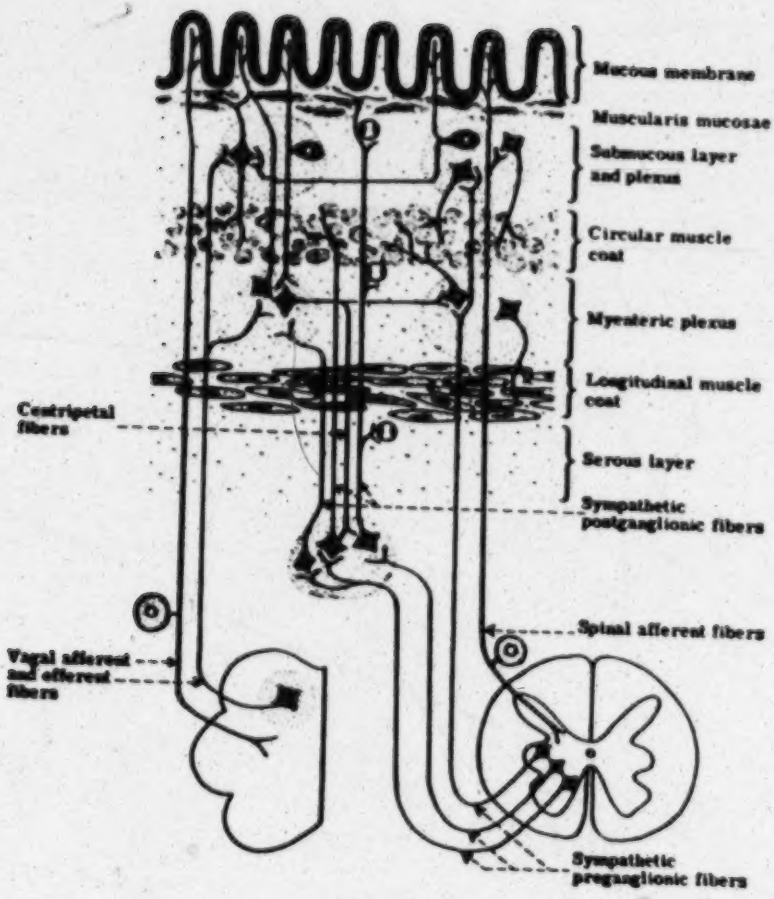

Figure 1: Diagramatic representation of the anatomy of the intestinal wall and its innervation (Schofield, 1968).

or circular direction, the deviation was randomly clockwise or anti-clockwise. Schofield's observations (1968) support Elsen and Arey's views.

2. Innervation of Intestinal Smooth Muscle:

(a) Extrinsic Innervation:

The smooth muscle of the gastrointestinal tract is innervated by both the parasympathetic (excitatory) and the sympathetic (inhibitory) divisions of the autonomic nervous system (for review see Youmans, 1968; Daniel, 1973). The parasympathetic supply to the small intestine is furnished by the vagus nerve the efferent fibers of which are preganglionic and terminate on neurons of the ganglionated myenteric and submucous plexuses in the intestinal wall. Postganglionic fibers from these plexuses innervate the smooth muscle and secretory cells. The preganglionic sympathetic fibers innervating the small intestine originate mainly from T_9 and T_{10} and synapse in the celiac (duodenum) and superior mesenteric (jejunum and ileum) prevertebral ganglia. The postganglionic fibers from these ganglia reach the intestine along branches of the celiac (duodenum) and superior mesenteric (jejunum and ileum) arteries.

The gastrointestinal tract has a rich afferent innervation; afferent fibers are found in the same nerves containing the sympathetic and parasympathetic fibers. Afferent fibers from the duodenum enter T_6 to T_9 and those from the jejunum and ileum enter T_9 to T_{12} (Crosby, Humphrey and Lauer, 1962).

(b) Intrinsic Innervation:

This consists of the myenteric (Auerbach's) plexus which is located between the longitudinal and circular muscle layers of most of the bowel. The anatomy of Auerbach's plexus has been described in detail by Schofield (1968) (see also Daniel, 1973). Briefly, it consists of postganglionic cholinergic neurons identified by their cholinesterase staining properties and by the presence of small agranular vesicles at their varicosities. On these neurons terminate axons of postganglionic sympathetic fibers as well as axons of preganglionic parasympathetic fibers. The postganglionic cholinergic fibers from these ganglia as well as some postganglionic sympathetic fibers terminate on the smooth muscle cells. Generally, the adrenergic innervation is very sparse in the longitudinal muscle layer and is richer in the circular muscle layer. Adrenergic axons are identified by their fluorescence staining properties and by the small dense core vesicles in their varicosities, the electron dense material of which can be depleted by reserpine, 6-hydroxydopamine and related substances. Using these identification criteria no adrenergic neuron cell bodies were found in the mammalian gut except in the guinea-pig proximal colon (Costa, Furness and Gabella, 1971; Furness and Costa, 1971).

In addition to these cholinergic neurons and adrenergic axons, neurons with large granular vesicles which differ from those seen in small numbers in adrenergic and cholinergic nerves in that they are bigger (800-2000 Å) and do not have a prominent electrontransparent halo between the vesicle membrane and its granular core have been described in the myenteric plexus of a number of vertebrate species

(Hager and Tafuri, 1959; Thaemert, 1963; Tafuri, 1964; Taxi, 1965; Rogers and Burnstock, 1966a,b; Bennett, M. R. and Rogers, 1967; Nagasawa and Mito, 1967; Pick, De Lemos and Ciannella, 1967; Kubozoe, Diakoku and Takita, 1969; Pick, 1970; Baumgarten, Holstein and Owman, 1971; Burnstock and Iwayama, 1971). These are thought to be the fibers mediating the nonadrenergic inhibition in the gut which is elicited by vago-vagal reflexes or by vagal stimulation after atropinization in the stomach (Greeff, Kasperat and Osswald, 1962; Martinson and Muren, 1963; Paton and Vane, 1963; Martinson, 1965a,b; Jansson and Martinson, 1966; Bülbring and Gershon, 1967; Jansson, 1969a,b; Abrahamsson and Jansson, 1969; Ohga, Nakazato and Saito, 1969, 1970; Abrahamsson, Jansson and Martinson, 1971; Beani, Bianchi and Crema, 1971), by transmural stimulation in the small intestine (Holman and Hughes, 1965a,b; Day and Warren, 1967, 1968; Kuriyama, Osa and Toida, 1967a; Kosterlitz and Lyndon, 1969; Gonella, 1971; Weston, 1971) and colon (Holman and Hughes, 1965a; Goldenberg, 1968; Crema, Del Tacca, Frigo and Lecchini, 1968; Bianchi, Beani, Frigo and Crema, 1968; Furness, 1969a,b, 1970; Bianchi, Beani and Crema, 1970; Costa and Furness, 1971; Rikimaru, Fukushi and Suzuki, 1971; Small, 1972) and by pelvic nerve stimulation in a limited distal portion of the guinea pig colon [Costa (quoted by Burnstock, 1972)].

The central and afferent connections of nonadrenergic inhibitory neurons are not yet established. In the stomach and distal rectum they are clearly postaganglionic and connected to the vagal and pelvic nerve trunks respectively. In the colon no inhibition could be elicited upon pelvic nerve stimulation (Garry and Gillespie, 1955;

Rand and Ridehalgh, 1965; Gillespie, 1968; Hultén and Jodal, 1969; Furness, 1969a, 1970; Bianchi, Beani and Crema, 1970). Thus it would appear that the nonadrenergic inhibitory neurons in the colon are completely intramural and it is likely that they may be involved in enteric reflex activity (Burnstock, 1972). It has not been determined whether the inhibitory nerves in the small intestine are controlled by preganglionic parasympathetic fibers mainly because the pathways of vagal fibers to different segments of the intestine are unknown (Burnstock, 1972). At present the only known way of producing nonadrenergic inhibition in the small intestine is transmural stimulation.

Studies on the nature of transmitter released by the preganglionic fibers to these neurons in the stomach and taenia coli implicate both acetylcholine and 5-hydroxytryptamine since their activity was partially inhibited by either nicotinic or 5-HT blocking agents (Drakontides and Gershon, 1971). Thus the transmitter to these neurons has been suggested to be acetylcholine for some (Greeff, Kasperat and Osswald, 1962; Paton and Vane, 1963; Martinson, 1965a,b; Campbell, 1966; Ohga, Nakazato and Saito, 1970) and 5-HT for other fibers (Bülbring and Gershon, 1967; Drakontides and Gershon, 1971). However, while Beani, Bianchi and Crema (1971) confirmed the existence of a small component of hexamethonium-resistant vagal inhibition, they were unable to repeat the experiments that were taken as evidence that this was due to the presence of tryptaminergic synapses in the vagal pathway (see also Nakazato and Ohga, 1971). Furthermore, Martinson (1965b) was unable to mimic vagal relaxation of the stomach by 5-HT and fluorescence histochemical studies of mammalian vagal nerve trunk failed to

show more than a few fluorescent fibers supplying the stomach (Muryobayashi, Imori and Fujiwara, 1968; Nielsen, Owman and Santini, 1969). The transmitter released from nonadrenergic inhibitory neurons has been suggested on strong evidence to be adenosine triphosphate (ATP) or adenosine diphosphate (ADP) (Satchell, Burnstock and Campbell, 1969; Burnstock, Campbell, Satchell and Smythe, 1970; Su, Bevan and Burnstock, 1971). Burnstock recently reviewed the evidence that ATP is the transmitter released from these neurons for which he coined the name "purinergic nerves" (Burnstock, 1972).

B. Historical Highlights:

The earliest studies on the electrical activity of the gastrointestinal tract were those of Alvarez and his associates in the 1920s (for a review see Alvarez, 1940). They were able to record cyclic potential fluctuations from the small intestine of rabbits, cats and dogs using extracellular calomel electrodes connected to an Einthoven string galvanometer with a long time constant (Alvarez, 1924; Alvarez and Mahoney, 1922a,b, 1924). Their studies clearly showed that electrical oscillations could be recorded in both intact and isolated segments of the small bowel. They noted that these oscillations were related to the mechanical activity and had the same rate as rhythmic contractions. In addition, a gradient of frequency of these electrical oscillations similar to that known for rhythmic contractions (Alvarez, 1914, 1915, 1918) was found along the intestine. Vagal stimulation failed to alter the electrical activity while stimulation of the splanchnic nerve or or topical application of adrenaline inhibited motility but electrical

oscillations remained unaltered. The demonstration of electrical waves in the absence of contractile activity led Alvarez and Mahoney (1924) to "think of an automobile with the engine running and clutch out. From time-to-time some mechanism lets 'clutch' in and motion appears."

During the 1930s, the findings of Alvarez were confirmed by Berkson (1933a-d), Puestow (1932, 1933) and Castleton (1934) who conducted their researches using essentially the same techniques. Berkson (1933b) studied the origin of the potential variations in the intestine and concluded that the dissociation of electrical from mechanical activity "militates against the view that the potential changes originate as muscle action currents" as Alvarez originally thought and he put forward the hypothesis that they "reflect rhythmic pulsations that emanate from the intrinsic plexus in the intestine." Although the galvanometers used in all these studies had long time constants which probably could not record faithfully muscle action potentials during mechanical activity, a careful inspection of some of Berkson's figures showed that spikes were sometimes recorded. However, these spikes were ignored and attention was devoted chiefly to the slow potential changes which followed fairly closely the mechanical activity.

In the late 1930s, Bozler (1938) introduced the use of new amplifiers with an improved frequency-response range into smooth muscle electrophysiology. With this technique the electrical activity of intestinal muscle was shown to consist of spikes in addition to the slow potential variations and the contractile activity was associated with the spikes and not with the slow electrical oscillations (Bozler, 1938, 1942, 1946). Based on his studies, Bozler (1942) visualized

that "the slow potentials...represent local potentials which initiate impulses. These potentials can be regarded as an expression of automaticity of the muscle. Whether they actually produce impluses naturally depends on whether muscular excitability is high enough for the conduction of impulses."

After the introduction of intracellular microelectrode recording techniques to smooth muscle electrophysiology by Bülbring and Hooton (1954), Greven (1954) and Woodbury and McIntyre (1954), it was soon applied in studies of the small intestine both in vivo (Daniel, Honour and Bogoch, 1960) and in vitro (Bortoff, 1961a; Nagai and Prosser, 1963a,b; Gonella, 1964 and 1965; Tamai and Prosser, 1966; Kobayashi, Nagai and Prosser, 1966). The use of this technique clearly showed that the electrical activity of the small intestine consists of two main components; the control activity or the basic electrical rhythm and the response activity or the spike potentials. Each complex of the electroenterogram consists of a control potential or a slow wave on which one to a burst of several spikes may be superimposed, each spike is preceded by a small prepotential.

It must be emphasized here that the "technical difficulties due to the small size (2.5 μ in diameter) and to the spontaneous mechanical activity of the muscle cells have prevented a more widespread use of this (intracellular electrode) technique" (Prosser and Bortoff, 1968). It is because of these difficulties that most workers in this field resorted to a variety of extracellular recording techniques both in vivo and in vitro which have successfully yielded valuable information on the electrical activity of the gastrointestinal tract and its modulation by neural or chemical influences.

C. The Myogenic Electrical Control Activity:

When microelectrodes are inserted into cells of the longitudinal smooth muscle layer of the small intestine, spontaneous rhythmic sinusoidal monophasic oscillations which represent changes in membrane potential can be recorded. These waves arise from a membrane potential of 30-50 mV, their amplitude varies from 5-15 mV and their duration (the period from the onset of depolarization phase to the end of repolarization phase) is about 2 seconds and their frequency depends on the anatomical location from which the recording is made (see below) and the species of animal studied, but is surprisingly constant for the same preparation. Such oscillations of the membrane potential have been recorded by intracellular electrodes from the small intestine of most species including dog (Daniel, Honour and Bogoch, 1960), cat (Tamai and Prosser, 1966, Kobayashi, Nagai and Prosser, 1966; Kobayashi, Prosser and Nagai, 1967), rabbit (Bortoff, 1961a; Gonella, 1964) and guinea pig (Hukuhara and Fukuda, 1968). They have been referred to as action currents (Alvarez and Mahoney, 1922a,b), sustained potentials (Bozler, 1946), slow potential A (Ambache, 1947), basic electrical rhythm or BER (Bass, Code and Lambert, 1961), pacesetter potentials (Code, Szurszewski, Kelly and Smith, 1968) and control potentials (collectively, control activity) (Daniel, 1969).

1. Relation of Control Activity to Response Activity and Response Activity to Motility:

It is clear from the work of Bass and his associates (Bass, Code and Lambert, 1961; McCoy and Bass, 1963) that in the small

intestine the control potentials, spike activity and mechanical activity bear a 1:1:1 temporal relationship to each other when all are present. There is now a consensus of opinion that the control potential depolarization and repolarization phases alternately increase and decrease the excitability of the smooth muscle cell membranes, respectively. Prepotentials appearing on the depolarization phases and peaks of the control potentials serve as direct triggers for spiking. The spikes associated with a control potential produce a contraction. Irrespective of the number of spikes on each control potential, there is only one contraction per control potential, the force of which may be related to the number of spikes per slow wave. Some control potentials can occur without spiking; their inability to initiate spikes may reflect lower excitability of the smooth muscle membrane. The relationship between spikes and contractions had been thoroughly discussed by Bass (1968). There has been no clear identification of any specific type of contractile activity (segmentation, peristalsis, pendular movement) with any specific pattern of spiking. Spikes can occur out of phase with the slow waves under certain conditions in which case they may be associated with a spasm (e.g., after administration of morphine) or with a ring of contraction induced by stroking the intestine (Daniel, Wachter, Honour and Bogoch, 1960).

Studies on the contractile activity of the dog small intestine <u>in vivo</u> showed that three patterns could be observed; namely, basal, burst and intermediate types (Reinke, Rosenbaum and Bennett, 1967). Basal activity consisted of low amplitude contractions with no appreciable change in tone. Burst activity interrupting the basal

pattern consisted of a burst of high amplitude contractions associated with an increase in basal tone. The intermediate activity consisted of varying amplitude ungrouped contractions often superimposed on a moderate increase in tone. In fasted dogs the burst activity lasted about 10 minutes; the basal activity lasted for 40 minutes and the intermediate activity about 10 minutes. On the other hand the durations of these activities were 1, 15 and 44 minutes respectively in fed dogs. The burst activity migrated from the upper duodenum to the terminal ileum. In his study of the interdigestive electrical activity in fasted dogs, Szurszewski (1969) uncovered the basis of these patterns. He described a "migrating electric complex" consisting of a consecutive series of control potentials on each of which is superimposed a burst of large amplitude spikes. The complex develops in the upper duodenum and migrates caudally to reach the terminal ileum in about 80-180 minutes by which time another complex was developing in the duodenum and proximal ileum. The duration of the complex at all levels of the bowel was 5-7 minutes. The precomplex electrical activity consisted of a period characterized by control activity without spikes (basal contractile activity) followed by a period of randomly occurring bursts of spikes associated with some control potentials (intermediate activity) with the bursts becoming more frequent and spikes larger in amplitude. The period terminated when, as every control potential displayed large spikes, a group of them amalgamated into a recognizable complex which then migrated caudally in an orderly fashion (burst contractile activity). This phenomenon has also been observed in sheep and rabbits (Grivel, 1971). The motility correlates of this interdigestive myoelectric

complex were studied recently in the dog (Grivel and Ruckebusch, 1972; Code and Schlegel, 1973), rabbit and sheep (Grivel and Ruckebusch, 1972). In these studies the complex was associated with strong segmental contractions (burst contractile activity) which appear to serve a propulsive function.

2. The Origin of the Control Activity:

There had been strong disagreements as to whether the control activity is myogenic or neurogenic. While Alvarez and Mahoney (1922b) believed that the control activity (which they referred to as "smooth muscle action currents") was myogenic in origin, Berkson concluded that "the rhythmic electrical changes reflect rhythmic pulsations that emanate from the intrinsic nerve plexus of the intestine and that these have a regulating function in the physiology of the small bowel" (Berkson, 1933b). A third view was that held by Ambache (1947) and which suggested that the control activity originates in the "nerve net which is present round Auerbach's plexus and in the interstices of the smooth muscle." The 'nerve net' he referred to is a network of interstitial cells interweaving among the neural elements in the intestine and sometimes forming close relationships with muscles. Since their discovery by Cajal (1893) these interstitial cells were thought to be nerve cells (see Boeke, 1949) until they were shown by the electron microscope to be composed primarily of fibroblasts (Richardson, 1958; Taxi, 1965; Rogers and Burnstock, 1966a).

However, current evidence strongly indicates that the control activity is a myogenic phenomenon. Isolated segments of the intestine

continue to show their inherent rhythmic contractions (Berkson, 1933a) and exhibit electrical control and response activities (Bortoff, 1961a; Tamai and Prosser, 1966) in the absence of extrinsic innervation. The rhythmic activity was found to persist after the application of a variety of drugs which are known to interfere with transmission of nerve impulses. These drugs include procaine (Holaday, Volk and Mandell, 1958), tetrodotoxin (Liu, Prosser and Job, 1969), ganglion blocking drugs (Ambache, 1947; Milton, Smith and Armstrong, 1955; Holaday, Volk and Mandell, 1958; Daniel, Wachter, Honour and Bogoch, 1960), cholinergic (muscarinic) and adrenergic blocking agents (Daniel, Wachter, Honour and Bogoch, 1960) and reserpine (Daniel and Bogoch, 1958; Daniel, 1965, 1968). Procedures which are believed to lead to the destruction of ganglion cells in isolated preparations such as storage at low temperature for several days or hypoxia also did not abolish rhythmic activity (Hukuhara, Kotani and Sato, 1962; also see Prosser and Bortoff, 1968). Furthermore, action potentials recorded from nerve cells in the myenteric plexus bear no direct relation to the activity of intestinal smooth muscle (Yokoyama, 1966; Wood, 1970; Ohkawa and Prosser, 1972). All these findings present strong evidence against the neurogenic origin of the control activity and support the concept that this activity is a myogenic phenomenon.

The next question related to the origin of the control activity was, from which muscle layer do they originate? Daniel, Honour and Bogoch (1960) conducted an <u>in vivo</u> microelectrode study on dog small intestine the results of which suggested that "possibly the occurrence of periodic slow waves is an inherent feature of the

longitudinal muscle of the intestine." Further studies showed conclusively that the control activity originates in the longitudinal layer and is electrotonically spread to the circular layer probably via strands of connecting muscle fibers. Isolated circular muscle preparations failed to show control activity (Sperilakis and Prosser, 1959; Bortoff, 1961a) while isolated longitudinal muscles showed control activity indistinguishable from that of the intact preparations (Bortoff, 1961a).

3. The Gradient of Control-Potential Frequency and Other Gradients:

Alvarez and Mahoney (1922a,b) were the first to record the intestinal control potentials and to observe that their frequency declined from duodenum to terminal ileum in anaesthetized dogs. A similar frequency gradient of the rhythmic contractions in different segments of the small bowel was already known to exist (Legros and Onimus, 1869; Alvarez, 1914 and 1915). The control-potential frequency gradient has since been repeatedly confirmed in isolated intestinal segments (Puestow, 1932), in the intact small intestine of anaesthetized dogs (Daniel, Carlow, Wachter, Sutherland and Bogoch, 1959; Diamant and Bortoff, 1969a,b; Sarna, Daniel and Kingma, 1970, 1971) and in the intact intestine of conscious dogs with chronically implanted electrodes (Bunker, Johnson and Nelsen, 1967; Nelsen and Becker, 1968; McCoy and Baker, 1969; Szurszewski, Elvebeck and Code, 1970) and in man (Christensen, Schedl and Clifton, 1964, 1966).

In anaesthetized dogs, the frequency of control potentials in the upper duodenum ranged from 15 - 21/min and remained constant throughout the duodenum and then progressively decreased in the jejunum and ileum reaching a frequency of 7 - 11/min in the terminal ileum (Daniel, Carlow, Wachter, Sutherland and Bogoch, 1959). Later Bunker, Johnson and Nelsen (1967) confirmed the existence of the frequency gradient in unanaesthetized dogs with chronically implanted electrodes. Their frequencies were a little higher than those reported by Daniel et al. (1959), an observation which might be due to the hypothermia produced by anaesthesia; cooling was shown to decrease the slow wave frequency (Daniel, Honour and Bogoch, 1960). Bunker et al. noted that the frequency remained essentially constant (frequency plateau) over the duodenum and proximal jejunum, but then decreased in an apparently "step-wise fashion"; i.e., the frequency showed other plateaus, from this point to the terminal ileum. The "step-wise" nature of the frequency gradient was confirmed in subsequent studies in anaesthetized animals (Diamant and Bortoff, 1969a and b; Sarna, Daniel and Kingma, 1970 and 1971) and in human subjects (Christensen, Schedl and Clifton, 1966). Diamant and Bortoff (1969a) reported the existence of multiple frequency plateaus in cat and dog intestine but specified them to be of variable length and frequency. Each frequency plateau was invariably separated from the next by an area of waxing and waning, the pattern of which could be reproduced by crossing the input leads from electrodes placed on the two adjacent plateaus. Sarna et al. (1970, 1971) however, found only one stable plateau in dogs' small intestine which extended over the entire duodenum and part of jejunum. The

wave forms recorded from successive electrodes (1 - 5 cm apart) in this plateau region were phase locked in the sense that they never fell 360° or more out of phase. Caudal to this plateau the control-potential frequency decreased progressively till the terminal ileum and showed a tendency to get phase locked, but then some control potentials fell 360° or more out of phase quite irregularly, thus preventing the formation of a stable plateau. The finding of one stable plateau corroborated a similar observation by Szurszewski et al. (1970). The apparent discrepancy between the results of Diamant and Bortoff and those of Sarna et al. may be, at least partly, due to the definition by Sarna et al. of the frequency plateau as not only the region over which the frequency is constant but also over which the wave forms are phase locked.

In isolated loops of rabbit small intestine, Alvarez in 1914 observed that the frequency of rhythmic contractions were lower the more caudal the origin of the segment. In the intact bowel of anaesthetized rabbits a gradient of rhythmic contractions was present, but the frequencies were faster than those of corresponding isolated loops at all levels of the bowel (Alvarez, 1915). Douglas (1948, 1949) demonstrated that in unanaesthetized dogs with Biebel loop preparations the frequency of contraction of a segment of the intestine is partly dependent on its continuity with orad bowel , for transection or clamping orad to the point of testing reduced the frequency of contractions but transection or clamping caudad did not. Clamping (Milton and Smith, 1956; Daniel, Carlow, Wachter, Sutherland and Bogoch, 1959) or sectioning and anasthmosis (Milton and Smith, 1956; Bunker and Nelsen, 1964; Bass and Wiley, 1965) of the duodenum significantly

decreased the frequency of control potentials distal but not oral to the clamp or anasthmosis. In a more detailed study of the effects of transection, clamping and heating (heating increases the control-potential frequency) of the distal duodenum of anaesthetized cats and dogs, Diamant and Bortoff (1969b) demonstrated the existence of an "intrinsic" frequency gradient observable after transection (or clamping) and characterized by lower frequencies than those recorded before transection. They concluded that an "unknown process in the upper end of the intestine serves to maintain the slow wave frequency of the initial plateau above its intrinsic level and that the frequency of each subsequent plateau is raised above the intrinsic frequency of its most proximal oscillator by the driving influence of its adjacent, oral plateau." This conclusion is supported by the studies of Grivel and Ruckebusch (1971) on the effects of transection and transplantation of the small intestine of cats and dogs with chronically implanted electrodes.

In a detailed study by Sarna, Daniel and Kingma (1971) on anaesthetized dogs, the frequency gradient was studied by recording from 53 electrodes implanted simultaneously along the intestine before and after section of the muscle layers at 15 cm intervals. They recorded intrinsic frequencies that were consistently well below the frequencies of the same locations before section. Furthermore, they showed that the gradient of intrinsic frequencies was invariably exponential rather than linear.

Thus, the characteristics of intestinal slow wave, namely, the existence of a frequency gradient with one or more frequency

plateaus, the demonstration of an intrinsic frequency gradient characterized by lower frequencies and the existence of regions of waxing and waning between frequency plateaus led to the assumption that intestinal smooth muscle may be regarded electrically as a series of loosely coupled relaxation oscillators having successively decreasing intrinsic frequencies (see below).

Other gradients:

After his demonstration of the existence of a gradient in the frequency of rhythmic contractions in the intestine, Alvarez (1919, 1928) sought to explain the basis of this mechanical gradient by means of a metabolic gradient. He found a decreasing sensitivity to cyanide poisoning along the tract from which he concluded that there was a metabolic gradient. More recently the oxygen consumption in different regions of cat small intestine was measured (Dorman and Steggerda, 1961). A decrease in oxygen consumption was found down the intestine which, however, was attributed to the mucosal layer rather than the muscle wall. Tissue levels of adenosine triphosphate (ATP) have also been shown to generally decrease in an aboral direction in combined sections of the entire wall of the intestine (Hanninen, Hartiala and Nurmikko, 1964). Serotonin levels also exhibit a diminishing gradient down the small intestine of cats, guinea pigs, mice and dogs (West, 1958; Garattini and Valzelli, 1965). It is not clear whether any of these gradients has a possible relation to the mechanical and electrical frequency gradients. A role for the serotonin gradient is unlikely since reserpine (a known serotonin releaser) does not influence the electrical activity of the small intestine.

Another suggestion for the mechanical and electrical gradients is that they correlate with the abundance of nerve cells in the intestinal plexuses. Estimates of nerve cells in the cat are 12,170 per cm^2 in the duodenum and 3,700 per cm^2 in the jejunum (Leaming and Cauna, 1961). However, in an ultrastructural study of the dog small intestine, Daniel, Duchon and Henderson(1972) reported that no notable differences in innervation were found between duodenum and upper jejunum and ileum.

Thus, there seems that there is a general consensus that the slow waves originate in the longitudinal muscle layer of the duodenum and upper jejunum but can originate elsewhere at lower frequencies when functional continuity along the intestine is lost. The first part of the duodenum acts as a pacemaker for the rest of the upper intestine; it apparently does so merely because its longitudinal muscle possesses the highest intrinsic frequency of spontaneous slow wave generation. The duodenal pacemaker also influences lower regions of the small intestine but with aborally decreasing ability.

4. Coupling and Conduction of Control Activity:

The early findings that the control activity could be recorded with large monopolar electrodes while the spikes were not as readily recorded suggested that the control potentials must be nearly synchronous for many lengths of cells. Dipolar recording techniques do not show up the control potentials as well as the monopolar electrodes unless one electrode makes contact with an inactive region or the two electrodes are a long way apart (Holman, 1968). Daniel and Chapman

(1963) found that if monopolar recording was made from several regions about 1 cm apart on the longitudinal axis of the small intestine, the control potentials were found to be out of phase by time intervals proportional to the distance between the recording electrodes. They estimated the apparent conduction velocity of the control potentials along the small intestine of anaesthetized dogs. In the duodenum the control potentials travelled with an apparent velocity of 10-20 cm/sec. The phase difference as a function of distance increased progressively as the wave travelled aborally. The apparent conduction velocity of the control potentials in the jejunum and ileum were 6-10 cm/sec and 0.3-1.0 cm/sec respectively. These observations confirmed the earlier findings of Armstrong, Milton and Smith in 1956 and were recently corroborated by the results of Code, Szurszewski, Kelly and Smith (1968) and McCoy and Baker (1969) in conscious dogs with chronically implanted electrodes. The propagation of control activity in the circular direction appears to be extremely fast. When electrodes are placed around the intestine the control potentials recorded from all electrodes occur almost simultaneously (Daniel and Chapman, 1963).

The mechanism by which the conduction of the control activity occurs is not clear at the present time. Propagation can be visualized to occur by either a nervous mechanism, by mechanical pull (mechanical pull is excitatory to visceral smooth muscle), by chemical transmission or by electrical conduction (see Prosser and Bortoff, 1968).

Propagation was shown to occur independent of nerve activity. This is indicated by the finding that ganglionic (nicotine and hexamethonium) and cholinergic blocking agents (atropine and methanthyline)

in amounts sufficient to block the cardiovascular effects of vagal stimulation did not prevent the normal sequential appearance of slow waves at distal sites (Holaday, Volk and Mandell, 1958; Daniel, Wachter, Honour and Bogoch, 1960). Hence, the propagation of the control activity does not involve nervous pathways in the myenteric plexus containing cholinergic synapses or endings. A variety of evidence has also been presented suggesting that extrinsic nerves are not essential for the distal spread of the control activity (Milton and Smith, 1956; Holaday, Volk and Mandell, 1958 and Daniel, Wachter, Honour and Bogoch, 1960). Nor does mechanical activity seem to be involved since normal spread was shown to occur through regions that have been mechanically immobilized (Burnstock and Prosser, 1960). Prosser and Bortoff (1968) presented evidence against chemical transmission. They also presented arguments for electrical conduction between the smooth muscle cells. The ultrastructural basis of this type of transmission was suggested to be the nexus serving as a low-resistance pathway electrically connecting smooth muscle cells (see Barr and Dewey, 1968 for a review). The existence of nexal connections in the mouse (Lane and Rhodin, 1964) and rat (Oosaki and Ishii, 1964) intestinal muscle and in the circular intestinal muscle of the dog and cat (Dewey and Barr, 1962, 1964) has been reported.

However, in recent studies (Henderson, Duchon and Daniel, 1971; Daniel, Robinson, Duchon and Henderson, 1971; Daniel, Duchon and Henderson, 1972), the longitudinal layer of the dog duodenum was found devoid of nexuses which were found only in the circular layer while another type of cell contacts was found largely in the longitudinal

layer and occasionally in the circular one. This type which was termed "intermediate contact" consisted of a region of parallelism of the cell membrane of two adjacent cells, increased cytoplasmic density in this region, an intercellular space of about 500 Å and a central dense line. The longitudinal muscle layer of the guinea-pig ileum has also been found devoid of nexuses (Gabella, 1972) despite good evidence for electrical coupling (Kuriyama, Osa and Toida, 1967b). The finding of Daniel and his coworkers may explain the poor propagation of the spikes in the longitudinal muscle layer (see page 34). The authors speculated that the control potentials may be propagated in the longitudinal muscle layer by current flow through the extracellular spaces at the intermediate contacts, the regions of closest apposition of cells. This view is consistent with the finding that current coupling rather than voltage coupling was required to duplicate the properties of the control activity in a model which visualizes the longitudinal muscle layer as consisting of a chain of bidirectionally coupled relaxation oscillators (Sarna, Daniel and Kingma, 1971). It is only in terms of such coupling that the synchronization of the control activity over long distances of the small intestine in vivo and that the non-decremental "conduction" of control potentials in isolated intact segments can be explained. This type of coupling would also explain the progressive decrease in propagation velocity, or more appropriately the increase in phase lag, in the aboral direction (Daniel and Chapman, 1963) since the model predicts that the phase lag is a function of the difference between the intrinsic frequencies of the coupled oscillators (see below)

The spread of the control activity from the longitudinal to the circular layer and within the circular muscle layer was studied by Bortoff (1965). He marshalled the following evidence to indicate that the spread is electrotonic; (1) monophasic control potentials could be recorded with pressure electrodes across a segment of intestinal wall separating two chambers filled with Tyrode solution. Since the amplitudes of these potentials were reduced in proportion to the thickness of circular muscle removed from the luminal side of the preparation, it was evident that the recorded potentials were developed across the circular rather than the longitudinal layer. Moreover, merely replacing that part of circular layer removed did not return the amplitudes of the recorded control potentials to their original level. (2) In flat preparations of intestinal muscle in which a section of the longitudinal layer was removed exposing a part of the circular layer, monophasic control potentials recorded from a strip of longitudinal muscle appeared to be in phase with those recorded laterally in adjacent circular muscle. (3) The amplitudes of monophasic control potentials recorded from circular muscle decreased exponentially with distance from the edge of the longitudinal strip.

However, in a study by Kobayashi, Nagai and Prosser (1966), Bortoff's findings were confirmed for the flat preparation, but the authors argued for active propagation of the control activity in cylindrical preparations. Their argument is based on their finding that: (1) there is a latency difference between summed slow waves recorded from longitudinal and adjacent circular muscle corresponding to a "propagation velocity" of 80 mm/sec in the circular muscle, and

(2) slow waves could be recorded laterally in adjacent circular muscle of cylindrical preparations over long distances as compared to flat preparations indicating a space constant greater than 10 mm. In a more recent study, Bortoff and Sachs (1970) re-addressed themselves to the same question. They argued that the latency differences reported by Kobayashi et al. may be a reflection of the "phase velocity" of the slow waves which would be a function of the slow wave frequency and the cable properties (time constant) if they are electrotonically transmitted. Using the data of Kobayashi et al., they calculated a phase velocity between 35 and 148 mm/sec showing that a propagation velocity of 80 mm/sec was not inconsistent with an electrotonic mode of transmission. Furthermore, they could not record slow waves from the circular muscle of cylindrical preparation for as long distances as those reported by Kobayashi et al. The exponential decay in amplitude with distance from the lateral edge of the longitudinal layer indicated a space constant of 2.3 mm which was the same as that for the flat preparation. These findings along with their observation that both the longitudinal and circular muscle layers showed periodic contractions at the same time (see also Bortoff and Ghalib, 1972) were marshalled in favour of the electrotonic nature of the spread of the control activity from the longitudinal to the circular layer.

While the abundant nexuses between circular muscle cells are believed to be the morphological correlate of electrotonic spread within the circular muscle layer (see page 25), the untrastructural basis of the spread of the control activity from the longitudinal to the circular layer is still unsettled. Kobayashi, Nagai and Prosser (1966) reported

on the presence of muscle strands running obliquely between the two layers in cat small intestine and thus suggested that it is via these strands that control potentials spread from longitudinal to circular layer. However, Gabella (1972) was unable to find any interconnecting strands in the guinea pig-ileum; but he demonstrated the existence of nexuses and desmosome-like attachments between the two layers. Similarly, Daniel and Duchon could not find any interconnecting muscle strands in the dog duodenum (Daniel and Duchon, unpublished observations). Gabella suggested that the electrotonic transmission from the longitudinal to the circular layer as well as between circular muscle fibers may occur via nexal contacts. However, the question of nexal transmission has recently been questioned by the finding that some smooth muscles for which there is good evidence for electrical coupling have only few or no nexuses. For example, in the vas deferens, there is good evidence of coupling (Tomita, 1967) but nexuses are rare (see Burnstock, 1970 for references). Similarly, no nexuses were found between uterine smooth muscle cells (Daniel and Garfield, to be published) although strong evidence for electrical coupling exists (Daniel and Lodge, 1973).

5. Coupled Relaxation Oscillator Models of the Control Activity:

Most of the characteristics of small intestinal electrical activity, particularly those relating to the slow-wave frequency gradient, the existence of frequency plateaus and the demonstration of an intrinsic frequency gradient characterized by lower frequencies were found to be reproduced by a model of a series of loosely coupled

relaxation oscillators* having successively decreasing intrinsic (uncoupled) frequencies (Nelsen and Becker, 1968; Diamant and Bortoff, 1969a and b; Diamant, Rose and Davison, 1971; Sarna, Daniel and Kingma, 1970 and 1971). The use of relaxation oscillators to simulate cardiac electrical activity (Van der Pol and Van der Mark, 1928) and cardiac sinus node activity (Roberge and Nadeau, 1966; Roberge, 1969) led to the suggestion that most biological rhythmic oscillations may be of the relaxation type (Nelsen and Becker, 1968).

Nelsen and Becker (1968) pointed out that the control activity of the small intestine showed close resemblance to a relaxation oscillator and may, therefore, be represented by the Van der Pol equation or a modification of this equation. Using an analog computer they evaluated some of the features of two loosely coupled relaxation oscillators of different frequencies. They concluded that their model was consistent with what was known about the frequency gradient, statistical variations of the rate and the coupling of the control activity to mechanical activity. Diamant, Rose and Davison (1971) studied the properties of a series of 25 Van der Pol relaxation oscillators with successively decreasing frequencies and which were loosely coupled by means of a digital computer. This model was succesful in reproducing most biological observations; (1) a step-wise frequency gradient with zones of

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*Relaxation oscillators produce oscillations which are characteristically: (i) aperiodic, (ii) easily synchronized or triggered (i.e., their frequency is readily entrained and shows frequency pulling), (iii) different in wave form from sinusoidal, (iv) described by a time period known as the relaxation time (see Van der Pol and Van der Mark, 1928).

waxing and waning of activity at the start of each frequency plateau, and (2) the degree of coupling between oscillators was the most important factor in determining how the intrinsic frequency gradient was manifest. Frequency plateau length, "frequency pulling" between adjacent frequency plateaus, simulated propagation velocity and the degree of waxing and waning of oscillator outputs were all affected by the degree of coupling. However, their results showed that coupling did not increase the frequency of the proximal (highest intrinsic frequency) oscillator above its intrinsic frequency (in in vivo experiments the frequency of the most proximal intestinal oscillator is higher than its intrinsic frequency). In the more recent study of Sarna, Daniel and Kingma (1970 and 1971) a chain of 16 oscillators with progressively decreasing frequencies and which were loosely coupled both in the forward and backward directions was used as a model. In this model both forward and backward couplings were necessary to simulate the characteristic properties of the intestine. They proposed two models which differ from each other only in the values of oscillator parameters and the manner in which each oscillator is coupled to its adjacent one. They showed that such coupling led to a higher coupled frequency of the most proximal oscillator than its intrinsic frequency. By adjusting the coupling ratios they could obtain one or more frequency plateaus. Other effects on the frequency and phase relationships of intestinal electrical activity following transection, constriction, or cutting of the longitudinal and circular muscle layers could also be reproduced by their models.

One general property of relaxation oscillators is that they can follow within limits the frequency of an appropriately applied

electrical stimulus. Specht and Bortoff (1972) tested this property in strips of longitudinally oriented muscle from the cat stomach, duodenum, jejunum and distal ileum in a Tomita-type apparatus. Depolarizing rectangular current pulses as well as sine waves were applied by large extracellular electrodes while the electrical activity was recorded by two chlorided silver wire electrodes placed at atout 4 and 10 mm from the stimulus respectively. Their results clearly indicate that both kinds of stimuli applied at a frequency higher than the spontaneous frequency of the preparation led to an increase in the frequency of slow waves which became entrained and the waves propagated away from the stimulated area. In all preparations the propagation velocity appeared inversely related to the slow wave frequency. This pattern continued as long as the stimulus was applied. Upon the termination of the stimulus, the original frequency, propagation velocity and direction of propagation resumed. The results of this study provided additional evidence that the small intestine (and the stomach) can be considered from an electrical view point as a chain (or array) of loosely coupled relaxation oscillators. They also lend support to the concept that the slow wave activity may be propagated along the intestine by local circuit current flow.

6. The Physiological Role of the Control Activity in the Motor Function of the Intestine:

The role played by the control activity in the regulation of the motor activity of the small intestine (and the stomach) can be visualized as a twofold one: (a) a pacemaker function: the control

potential provides a mechanism by which the membrane potential is periodically depolarized. This leads to periodic enhancement of the probability of spiking, and (b) a synchronizing and coordinating role: the characteristics of the spread of the control activity in both the longitudinal and circular axes provide the basis of this function. The extremely fast conduction in the circular axis provides the basis for simultaneous spiking in this axis while the slower conduction in the longitudinal axis causes a measurable phase lag in spiking longitudinally. Thus the contractile activity appears as a ring of contraction proceeding as a travelling wave front in the longitudinal axis of the gut moving normally in an aboral direction.

D. Prepotentials:

These are slow depolarizations varying between 1 and 6 mV which can be seen in intracellular records from small intestinal smooth muscle. They occur on the depolarizing phase or the peak of the control potential and are usually followed by spikes. The prepotentials apparently directly trigger the spikes; but sometimes they fail to do so, and they appear superimposed on the slow wave without spikes (Baker, 1969).

E. Spikes:

The spikes in the longitudinal smooth muscle of the small intestine appear during the depolarizing phase (or occasionally at the peak)of the control potentials. Each control potential may be associated with one up to several spikes; each control potential is associated

with one and only one phasic contraction. Spikes may be preceded by prepotentials. Isolated circular muscle of the cat small intestine was shown to produce spikes only (Prosser and Sperilakis, 1956).

In the whole animal the electrical complex consisting of the control potential, prepotentials and spikes corresponds to a segmental contraction (Daniel and Chapman, 1963). The force of contraction has been correlated with the number of spikes per control potential (Bass, 1968). Spikes out of phase with the control potentials have also been noted during a spasm (e.g., following morphine) and near a pulsating ring of contraction induced by stroking the intestine (Daniel, Wachter, Honour and Bogoch, 1960). However, in the intestine of unanaesthetized (Milton and Smith, 1956; Holaday, Volk and Mandell, 1958; Bass, Code and Lambert, 1961) or anaesthetized animals (Daniel and Chapman, 1963) out-of-phase spikes are extremely rare or absent. In contrast to the control potentials, spikes appear to be propagated only very short distances, if at all, and usually occur independently in number and pattern in different cells (Daniel and Chapman, 1963). The spike duration, like in other smooth muscles, is generally longer than in skeletal muscles being 12 msec in guinea-pig duodenum (Greven, 1956) and 42-48 msec in cat circular muscle (Nagai and Prosser, 1963a). The spikes may or may not overshoot. The rate of rise of intestinal spikes is 10-15 V/sec in cat duodenal circular muscle (Nagai and Prosser, 1963b). In most cases, each spike is followed by a transitory "overshoot" which may hyperpolarize the membrane beyond its resting potential but the membrane quickly recovers from this to complete the control potential or go into the next prepotential-spike overshoot sequence.

F. Modulation of the Myogenic Activity of the Small Intestine by Neural, Hormonal and Other Influences:

There are many studies which indicate that the myogenic electrical and motor activities of the gastrointestinal tract are subject to regulation by extrinsic and intrinsic neural activity as well as by endogenous chemicals including gastrointestinal hormones, other substances of gastrointestinal origin and hormones or humoral substances other than those from the gastrointestinal tract (see Daniel, 1973). In his conceptual analysis of gastrointestinal motor function, Daniel, 1973 suggested that the mechanisms by which the motor function may be modulated involve: (1) altering the intrinsic frequencies, (2) altering the mutual coupling between oscillators, (3) changing the coupling of control (slow-wave) activity to response activity or (4) changing the coupling between the response activity and the contractile system in smooth muscle cells.

1. Neural Influences:

(a) Extrinsic Nerves:

Removal of extrinsic nerve influence has no significant effects on the electrical and motor activity of the small intestine (Berkson, 1933a; Puestow, 1932; Bortoff, 1961a). In a recent study, Marik and Code (1973) observed that vagotomy reduced the frequency of occurrence of the interdigestive myoelectric complexes in fasted conscious dogs. This effect may reflect a neural influence on the coupling between control and response activities. Electrical stimulation of the vagal

nerve supply to the small intestine of cats (Van Harn, 1963) and rabbits (Gonella, 1964) in vivo caused a slight depolarization which made the control activity more effective in eliciting response activity; the spiking frequency increased and contractions became stronger. Sympathetic nerve stimulation hyperpolarized the smooth muscle cell membrane and inhibited response activity and contractions (Van Harn, 1963). However, the responses to nerve stimulation depended on the state of muscle activity; vagal stimulation of a hyperactive intestine produced opposite effects to those usually observed and sympathetic stimulation of a quiescent intestine had paradoxical effects (Van Harn, 1963). Neither vagal nor periarterial nerve stimulation had any significant effect on the control activity.

(b) Intrinsic Nerves:

The role of the intrinsic nerves in the regulation of small intestinal motor function is poorly understood mainly because no technique is available to selectively damage the intrinsic nerve plexuses (see Daniel, 1968; Szurszewski and Steggerda, 1968; Khin and Daniel, 1970). The effects of stimulation of the intrinsic nervous system have little been studied in the small intestine. In the stomach (Paton and Vane, 1963; Beani, Bianchi and Crema, 1971) and colon (Gillespie and MacKenna, 1960; Burnstock, Campbell, Bennett and Holman, 1964) stimulation of intrinsic neurons produced one or a combination of two responses; an inhibitory response leading to muscle relaxation and an excitatory response leading to contraction. The inhibitory response could be unmasked after muscarinic receptor blockade and was shown to

result from stimulation of nonadrenergic inhibitory neurons the transmitter released by which is ATP (see review by Burnstock, 1972). The existence of a nonadrenergic inhibitory system in the small intestine of the guinea pig, mouse, rat, rabbit and cat has been demonstrated (see Burnstock, 1972 for references).

Intrinsic nerve stimulation in a number of gastrointestinal smooth muscles (Bennett, M. R., Burnstock and Holman, 1963, 1966; Bülbring and Tomita, 1967; Furness, 1969a,b, 1970) causes a transient depolarization (excitatory junction potential or EJP) in some cells or a transient hyperpolarization (inhibitory junction potential or IJP) in others. Some cells respond with an EJP followed immediately by an IJP. After muscarinic blockage the EJPs are abolished and only IJPs could be seen. Both responses can be blocked by tetradotoxin. The IJP was shown to be due to an increase in the potassium permeability of smooth muscle cell membranes (Bennett, M. R., Burnstock and Holman, 1963; Bennett, M. R., 1966; Tomita, 1972).

The intrinsic nonadrenergic inhibitory system may be responsible for the descending inhibitory reflexes in the gastrointestinal tract (see Furness and Costa, 1973; Abrahamsson, 1973).

2. Hormonal Influences:

The gastrointestinal tract is a source of a number of polypeptide hormones including gastrin, secretin, cholecytokinin, enterogastrone and motilin. These hormones exert profound effects on gastrointestinal motility. The available literature on the localization, release and possible physiological role of gastrin, secretin

and cholecystokinin in the modulation of gastrointestinal motor function has been recently reviewed (Daniel, 1973). The most recently discovered hormone, motilin, is a polypeptide present in the duodenum and is released upon alkalinization (Brown, Johnson and Magee, 1966; Brown, 1967; Brown, Mutt and Dryburgh, 1971; Brown, Cook and Dryburh, 1973). It stimulates motor activity in both antral and fundic gland area pouches of dog stomach. At present, the loci, mechanisms of action and possible physiological functions of these hormones in the regulation of gut motility are not convincingly established.

3. Other Chemical Influences:

Naturally occurring substances, other than neurotransmitters and gastrointestinal hormones are capable of influencing the motor function of the gastrointestinal tract. These include serotonin (5-hydroxytryptamine or 5-HT), histamine, bradykinin and the prostaglandins. Their effects on gastrointestinal motility have been recently reviewed (Bennett, A., 1970; Daniel, 1973). However, it is not known whether any of these substances plays some physiological or pathophysiological role in the regulation of motor activity in the tract.

CHAPTER 2

IONIC BASIS OF SMALL INTESTINAL ELECTRICAL ACTIVITY

A. The Resting Membrane Potential:

Generally speaking, cell membranes show three important characteristics: (i) an electrical potential difference, the resting membrane potential (RMP) can be measured across cell membranes; the inside of the cell being negative with respect to the outside; (ii) the cell membrane separates an intracellular solution with high potassium and low sodium from an extracellular solution with high sodium and low potassium; and (iii) the cell membrane is permeable to ions to varying degrees; it is usually most permeable to K and Cl ions and least permeable to Na ions.

Several theories have been proposed to explain these electrical and chemical gradients across cell membranes. Ling (1962) and Troshin (1966) suggested that these gradients result from selective adsorption of K ions to fixed negative charges within the cells (see Jones, 1970 for a reference). However, the most widely accepted theory is that originally developed by Bernstein (1912) as later modified by Goldman (1943). According to the Goldman version of this theory, the cell membrane is permeable to K, Cl and Na ions and the existence of concentration gradients for these ions leads to the developemnt of a diffusion electrical potential difference across the cell membrane

given by the Goldman equation.*

$$E = \frac{RT}{F} \ln \frac{P_K[K]_o + P_{Na}[Na]_o + P_{Cl}[Cl]_i}{P_K[K]_i + P_{Na}[Na]_i + P_{Cl}[Cl]_o}$$

where E is the membrane potential, R is the gas constant, T is the absolute temperature, F is the Faraday constant, P_K, P_{Na} and P_{Cl} are the membrane permeabilities to K, Na and Cl ions respectively, $[\ ]_o$s are the molar extracellular concentrations of ions and $[\ ]_i$s are the internal concentrations of ions.

The maintenance of the ionic gradients and hence of the potential gradients under conditions in which the membrane is permeable to these ions is only possible if some active mechanism operates to extrude the sodium ions that enter the cell under the influence of the sodium electrochemical gradient and to readmit the K ions that leak out down the K concentration gradient. Such a pumping mechanism has been first suggested by Dean (1941) and subsequently shown to exist in neuronal membranes and muscle cell membranes (see Caldwell, 1968 for review). The pump extrudes Na and accumulates K by a metabolically driven mechanism utilizing ATP as its energy source and is intimately related to a $Na^+ + K^+$ stimulated (ouabain sensitive) membrane adenosine triphosphatase (Skou, 1965; Caldwell, 1968). In the development of Goldman's equation

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*The assumptions used in the derivation of this equation are, (i) that the electrical field is constant throughout the membrane thickness, (ii) that the activity of an ionic species (Na, K or Cl) in the immediate vicinity of either side of the membrane is directly proportional to that of the aqueous solution on that side, (iii) that the activity coefficient for an ion is the same on both sides of the membrane, (iv) that the membrane is homogeneous, (v) that in equilibrium conditions there is no net current crossing the membrane (i.e., total membrane current = zero) and (vi) the operation of Na - K exchange pump does not contribute to the membrane potential or membrane current.

it was assumed (among other assumptions) that the activity of this sodium - potassium exchange pump (or simply the sodium pump) does not provide a direct contribution to the transmembrane potential; i.e., the pump is electroneutral. Such a condition can only be fulfilled if the pump extrudes one Na ion for every K ion it accumulates (a coupling ratio of 1) and thus its operation does not cause a net transfer of charges across the membrane.

However, studies on the electrogenicity of the Na - K exchange pump in a wide variety of tissues showed that the pump provides a direct contribution to the transmembrane potential at least under certain conditions. Such a pump is called an electrogenic pump and its electrogenicity can only be understood if it extrudes more sodium than it accumulates potassium (its coupling ratio is >1) and hence its operation leads to a net transfer of charges across the membrane; i.e., to a net outward current. The list of the tissues in which the pump was shown to be electrogenic is constantly growing and includes certain nerve cells and axons (Kerkut and Thomas, 1965; Nicholls and Baylor, 1968; Carpenter and Alving, 1968; Rang and Ritchie, 1968a; Thomas, 1969; Carpenter, 1970; Gorman and Marmor, 1970), skeletal muscle (Kernan, 1962; Cross, Keynes and Rybová, 1965; Adrian and Slayman, 1966), cardiac muscle (Page and Storm, 1965; Tamai and Kagiyma, 1968; Vassalle, 1970) and the smooth muscles of the uterus (Daniel, Paton, Taylor and Hodgson, 1970; Daniel, Robinson, Kidwai, Wolowyk, Taylor and Paton, 1971; Taylor, Paton and Daniel, 1969, 1970, 1971) and taenia coli (Casteels, Droogmans and Hendrickx, 1971a, 1973a,b; Tomita and Yamamoto, 1971). The subject of the electrogenic sodium pump has been recently reviewed by Ritchie (1971), Kerkut and York (1971) and Thomas (1972).

Up to the present, the author is not aware of any studies on the ionic basis of the resting membrane potential in the smooth muscle cells of the small intestine. However, there had been a number of studies on this subject on the guinea pig taenia coli smooth muscle, the most recent of which are those of Casteels (1969) and Brading (1971). In both studies, the tissue ion contents and the extracellular space were measured and the intracellular ionic concentration were calculated. The unidirectional Na, K and Cl ion fluxes were measured by radioactive tracer techniques and values for the membrane permeability to Na, K and Cl ions were computed. From the values of extra- and intracellular ionic concentrations and ionic permeabilities, a value for the membrane potential was calculated by the Goldman equation and was compared to the membrane potentials measured by intracellular microelectrodes. It is to be emphasized that these calculations are based on many assumptions regarding ion binding, ionic concentrations and activities in the vicinity of the cell membrane and the number and nature of intra- and extracellular ion compartments which makes the interpretation of the results difficult.

Nevertheless, using this approach, Casteels (1969) obtained the following values for the permeability constants: $P_K = 11 \times 10^{-8}$ cm/sec, $P_{Cl} = 6.7 \times 10^{-8}$ cm/sec and $P_{Na} = 1.8 \times 10^{-8}$ cm/sec. Using these values, he obtained a calculated membrane potential of -37 mV which was 15–20 mV lower (less negative) than the measured membrane potential. He concluded that this discrepancy between the measured membrane potential and the calculated one may be the result of the operation of an electrogenic sodium pump in this tissue. The author

compared the permeability constants he obtained with the values given for skeletal muscle and observed that the Na permeability was not significantly different in both muscles while the K and Cl permeabilities were much lower in taenia coli than in skeletal muscle. Thus, he concluded that the low membrane potential in smooth muscle cells may be caused by the low value of P_K and not a high value of P_{Na}. The low P_K may also explain the earlier finding that the maximal slope of the curve representing the change in the resting potential as a function of the logarithm of external potassium concentration was only 35 mV for a tenfold change of $[K]_o$ instead of the theoretical value of 61 mV for a membrane selectively permeable to K (Casteels and Kuriyama, 1966).

In a later study, Brading (1971) examined several models based on different assumptions as to the distribution of ions in taenia coli smooth muscle. In particular she assumed that only the slowest components of the efflux curves for Na, K and Cl represented transmembrane movements and compared the predictions of these models with respect to the membrane properties with available electrophysiological knowledge. She concluded that reasonable predictions of membrane properties could only be made using models in which the majority of the rapidly exchanging sodium was extracellular. Using one such model she obtained the following values for ion permeability constants: $P_K = 6.71 \times 10^{-8}$, $P_{Cl} = 4.4 \times 10^{-8}$ and $P_{Na} = 0.066 \times 10^{-8}$. Using these values she calculated a Goldman diffusion potential of -57 mV which is well in agreement with the measured values of -51 to -56 mV. She concluded that "although neither of the models described in this paper precisely predicts the membrane parameters derived from electrophysiological experiments,

nevertheless they suggest that these properties could be predicted from the passive membrane properties and distribution of ions, without having to postulate any specific mechanisms such as electrogenic pumping to account for the experimental results." However direct evidence for the operation of such a pump in taenia coli exists (Casteels, Droogmans and Hendrickx, 1971a, 1973a,b).

In a study by Tamai and Prosser (1966) aiming at differentiating the slow waves and spikes in longitudinal muscle strips of cat upper intestine it was found that the membrane potential was relatively insensitive to changes in external Na concentration while the curve relating the resting potential to external K concentration had a slope of about 30 mV per tenfold change in potassium concentration. In these respects the small intestinal longitudinal smooth muscle resembles other smooth muscles studied. However, the numerous electrophysiological and pharmacological differences between the longitudinal muscle of the colon and that of the small intestine and those between the gastrointestinal tract of the guinea pig and other species (Daniel and Chapman, 1963) stand as strong objections to extrapolation from the guinea pig to other species and from the colon to the small intestine. In this context, the need for studies on the ionic basis of the resting membrane potential of small intestinal smooth muscle is strikingly apparent.

2. The Control Potentials:

The earliest studies on the ionic mechanisms underlying the control activity in longitudinal muscle of the small intestine were those of Daniel in 1962 and 1965. He investigated the effects of

altering the extracellular ionic concentrations, metabolic inhibitors and inhibitors of active transport in dog's small intestine in intra-arterial perfusions of segments of the small intestine while recording the electrical activity of the affected segment extracellularly *in vivo*. His results are summarized as follows. Perfusion of solutions with reduced sodium (sucrose used as substitute), potassium or chloride (nitrate, sulphate or methylsulphate used as substitutes) concentrations markedly altered electrolyte concentrations in intestinal muscle without significantly altering the control activity. However, when lithium ion was substituted for sodium ion the control potentials were seriously depressed. Perfusions of normal solutions containing ouabain, sodium fluroide or Na_2-EDTA also seriously depressed the control potentials. These substances, like lithium, are believed to be direct inhibitors of the active transport process. Iodoacetate and 2,4-dinitrophenol in concentrations sufficient to cause downhill ion movements had little depressant effect on intestinal control activity. Sodium cyanide or 1,10-phenanthroline depressed slow waves. Prior reserpinization of the dog largely prevented the effect of sodium cyanide and diminished the effects of lithium ion, ouabain, sodium fluoride and Na_2-EDTA. In earlier studies (Daniel, Wachter, Honour and Bogoch, 1960; Daniel, 1962), the control activity was found to be sensitive to temperature and hypoxia; both hypoxia and low temperatures profoundly reduced the control potential amplitude and frequency while at temperatures higher than body temperature the control potential amplitude was slightly enhanced.

Based on these observations, Daniel (1962, 1965) concluded that lower concentrations of inhibitors of the active transport process abolished the control activity by causing the release of catecholamines from intrinsic nerve endings in the intestine. The released catecholamines then depressed the control activity. In higher concentrations inhibitors of active transport depressed the control potentials by direct action, unaffected by prior reserpinization. The control potentials were, therefore, postulated to originate from the operation of an oscillatory electrogenic sodium pump.

In a later in vitro microelectrode study by Tamai and Prosser (1966) an attempt was made to differentiate the control potentials from the spikes in the longitudinal muscle of cat small intestine. Decreasing the extracellular sodium down to 17.6 mM (normal solutions contained 151.1 mM Na^+) by replacement with equi-osmotic amounts of sucrose, choline chloride or lithium chloride markedly reduced the control potential amplitude and frequency without appreciably affecting the resting membrane potential. Reduction of extracellular calcium concentration depolarized the membrane and greatly reduced the control potential amplitude and frequency. High potassium (4-10 times normal) depolarized the membrane and abolished the control activity.

More recently, Daniel's electrogenic sodium-pump oscillation hypothesis attracted the attention of Job and his coworkers. In the first of their series of papers (Liu, Prosser and Job, 1969) the ionic dependence of intestinal control activity was studied in vitro in segments of cat jejunum from which the mucosa and submucosa had been removed by both extracellular pressure electrode and intracellular

microelectrode techniques. The recordings, however, were illustrated chiefly from studies with pressure electrodes. Total replacement of sodium in the organ bath by Tris-chloride abolished the control activity after about 40-60 minutes without altering the resting membrane potential, the spikes or (up to the time of their disappearance) the frequency of the control potentials. Partial replacement of Na^+ by $Tris^+$ decreased the amplitude and rates of rise and fall of control potentials. When lithium chloride was substituted for sodium chloride, the control potential amplitude and frequency progressively decreased till they were abolished in 15-20 minutes (faster than in Tris-Tyrode). The spikes persisted at normal height after the control activity disappeared. Ouabain, in concentrations less than 10^{-6} M reduced the amplitude of, or abolished, the control potentials and depolarized the membrane by about 4 mV. Doses small enough to diminish the amplitude of, but not abolish, the control potentials also reduced their frequency. The control activity was insensitive to high doses of tetrodotoxin. These authors also reported that iontophoretic injection of Na (but not of K) ions into longitudinal muscle cells increased the control potential amplitude some 3.5 times. However, their record shows that the amplitude increased and decreased instantaneously at the beginning and end, respectively of Na injection and remained constant during the entire period of injection. According to Bortoff (1972), one would predict a more gradual change in the height of the control potential both at the beginning and the end of ion injection. In contrast to Tamai and Prosser (1966) findings, the authors claimed that omission of $CaCl_2$ from the Tyrode solution had no effect on the control potential

amplitude and frequency although the rates of rise and fall were depressed. Similarly, substitution of Na propionate for NaCl did not alter either the control potential amplitude or frequency or the resting membrane potential. The authors interpreted their data as supporting the hypothesis put forward by Daniel (1962 and 1965) that the "slow waves result from a rhythmic electrogenic sodium pump."

In the second paper, Job studied the labelled ionic fluxes associated with the control activity in similar preparations to those used in the first paper. He used a rapidly revolving carousel to collect the perfusate from the tissue perfusion chamber while recording the electrical activity with an extracellular pressure electrode (Job, 1969). Using this technique he was able to collect 15 radioactive fractions per control potential. Despite the inherent weaknesses in this technique, the possibility that not all portions of the segment oscillated in phase and his use of the entire muscle wall (the circular layer of which does not generate control activity), the results indicated that an increased sodium influx occurred during the depolarization phase while the efflux of sodium was maximal early in the repolarization phase. He also studied the effects of temperature and metabolic inhibitors on the control activity. The control potential frequency had a Q_{10} of 2.73 and the rates of rise and fall have similar temperature coefficients of 2.5. Furthermore, the rate of repolarization, but not the frequency, exhibited an optimum at 37° C. Anoxia and sodium cyanide but not iodoacetic acid abolished the control activity. Based on these results, Job postulated that the control potential depolarization phase may be attributed to passive sodium influx; i.e., an increase in sodium permeability while

the repolarization phase of the control potential may result from electrogenic sodium pumping. This hypothesis is difficult to reconcile with two of their findings: (a) the finding that the rate of rise and rate of fall of control potential both had a high Q_{10} of 2.5. One would predict from the hypothesis that the rate of repolarization must be more sensitive to temperature than that of depolarization, and (b) the finding that iontophoretic injection greatly increased the control potential amplitude. The new hypothesis would predict a decrease in amplitude due to the decrease in the concentration gradient for Na if control potential depolarization was due to increased sodium permeability.

In the third paper, Job addressed himself to the question of the mechanisms by which the increase in Na permeability leading to the control potential depolarization and the subsequent stimulation of the electrogenic Na pump to repolarize the membrane are triggered (Job, 1971). In his earlier paper (1969) he discussed the possibility that the pump oscillations may be controlled either by cyclical production of ATP by mitochondria or by changes in ATP/ADP ratio. Thus his approach which is subject to serious criticisms, was to study the effects of antibiotics which are known to interfere with mitochondrial K^+ uptake and of inhibitors of mitochondrial ATP synthesis on the amplitude and frequency of the control potentials recorded by pressure electrodes from a cylindrical preparation of cat jejunum longitudinal and circular muscle. The fundamental, but untested, assumption was made that the actions of these compounds in the doses used were due to their mitochondrial effects. Valinomycin, which stimulates, and monensin, which inhibits mitochondrial K uptake, both initially enhanced and then inhibited the control potential

amplitude at low concentrations but their effects on the frequency were variable, small and probably insignificant. Uncouplers of oxidative phosphorylation (dinitrophenol, pentachlorophenol and oligomycin) caused a dose-dependent decrease in amplitude accompanied by either no change or a slight increase in frequency of control potentials. The electron transport inhibitors antimycin A and thenoyl trifluoroacetone effectively reduced both the amplitude and frequency. Sodium cyanide, rotenone and sodium amytal inhibited the control potential amplitude but not the frequency. Anoxia caused a progressive reduction of the amplitude but the frequency was relatively stable and was slightly decreased only after about 50 minutes in N_2-CO_2 (95%-5%) Tyrode. Furthermore, Job claimed that membrane potential oscillations could be produced after antimycin A inhibition by adding electron donors (tetramethyl-p-phenylenediamine dihydrochloride followed by ascorbic acid). Job took the results of this investigation as suggesting that "mitochondrial oscillations (in ATP synthesis) do not appear to be responsible for driving the membrane oscillations" and proposed that the depolarizing phase is a consequence of a build-up in ATP concentration at the membrane which "turns on" an increase in sodium conductance. Sodium ions leak in and coincidentally ATP level is sufficient to support active extrusion of sodium ions (perhaps coupled with potassium ion uptake although no direct evidence is available). The pump depletes ATP to subthreshold levels again, the sodium conductance decreases, and the membrane potential returns to its "repolarized state." Based on this postulated mechanism the differential response of amplitude and frequency of some of the compounds used in this study could be explained. The hypothesis predicts that the amplitude is

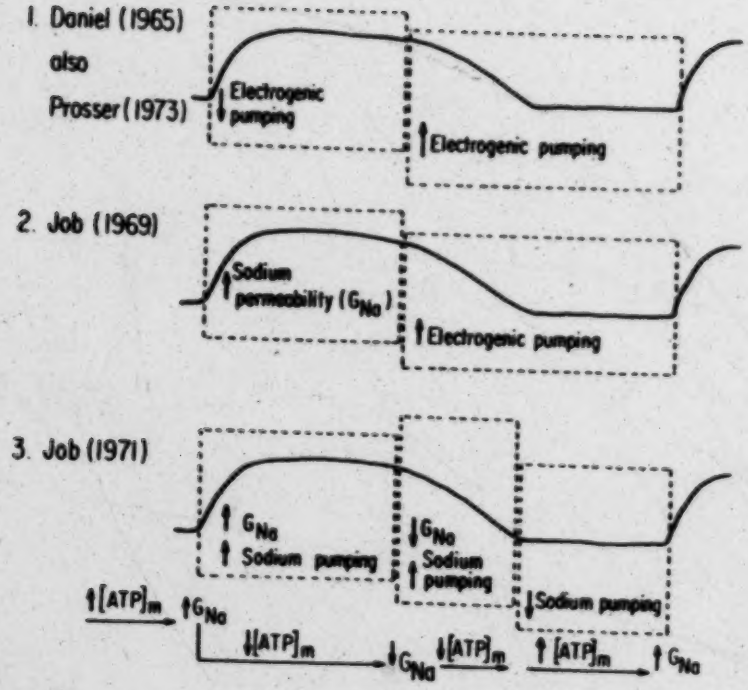

Figure 2: Postulated mechanisms of the ionic basis of the electrical control potentials of the longitudinal smooth muscle cells of the small intestine.

directly proportional to the ATP concentration at the membrane while the frequency would be related to the time required for ATP concentration to reach a threshold for activation.

In summary, there are at the present time three hypotheses which have been introduced to explain the ionic mechanisms responsible for the control potential generation (Fig. 2).

(1) The oscillating electrogenic sodium-pump hypothesis according to which the operation of an electrogenic Na pump contributes to the maximum resting membrane potential and the control potential results from turning off (depolarization) and on (repolarization) of this pump (Daniel, 1962, 1965).

(2) Job's hypothesis of 1969 which attributes the control potential depolarization to an increase in Na permeability and repolarization to electrogenic Na pumping. This hypothesis does not specify when does the increase in Na conductance turn off.

(3) The oscillating Na-permeability hypothesis suggesting that ATP-controlled oscillations in Na-permeability underlie the control potential (Job, 1971).

According to the sodium-permeability oscillation hypothesis, one would expect the control potential to be accompanied by changes in membrane resistance; a decrease during the depolarization phase and plateau and a return to the resting value during repolarization. Measurements of membrane resistance during intestinal control activity were performed in two laboratories using the double sucrose-gap technique. In the rabbit ileal longitudinal muscle strips, Mills and

Taylor (1971) recorded control potentials, spikes and prepotentials. They measured the 'electrotonic' potentials in response to hyperpolarizing current pulses at various phases of, and between, control potentials. A decrease in the size of the electrotonic potential was observed during the peak of the control potentials. From this finding the authors concluded that the membrane resistance decreases at the peak of the control potential and their results are consistent with the earlier hypothesis of Job (1969).

In the other study, of which only an abstract is so far available, (Weems, Connor and Prosser, 1973) no change in membrane resistance could be detected during control activity. Furthermore, the authors claimed that spontaneous inward-directed current transients were recorded when the membrane potential was clamped at its resting value, that these currents had the proper amplitude, duration and frequency necessary to produce control potentials, and that they were blocked by doses of ouabain which abolished the control activity. Since they could not detect changes in membrane resistance, they were led to the conclusion that the ionic currents responsible for the control potential need not result from ionic conductance changes but may result from a cyclical electrogenic sodium pump. Again, the absence of measurable resistance changes during control activity in this study is inconsistent with Job's ATP-dependent oscillating permeability model. The inherent difficulties and weaknesses in the use of double sucrose-gap techniques to clamp tissues with complex geometries (see Anderson, 1969; Johnson and Lieberman, 1971), and the finding that one of the essential criteria for the successful use of this approach; i.e, the ability

of the applied current to travel solely in the intracellular compartment of a node consisting of many cells, is not fulfilled in the longitudinal smooth muscle of the small intestine of dogs (Henderson, Duchon and Daniel, 1971) and probably other species brings the meaningfulness of the results of such experiments under serious doubts and questions. Nevertheless, the details of the latest study by Weems, Connor and Prosser (1973) will be awaited with interest.

As Bortoff (1972) pointed out, an equally serious criticism of Job's model is that it does not provide for a recently discovered property of intestinal control activity; namely, their ability to be electrically driven and entrained by appropriately applied depolarizing pulses both in double sucrose-gap (Mills and Taylor, 1971) and in Tomita-type experiments (Specht and Bortoff, 1972). One of the interesting findings of Specht and Bortoff is that depolarizing current pulses initiated control potentials immediately whereas hyperpolarizing pulses stimulated at the end of the pulse (an anodal break response). Based on this observation, the authors argue that "since depolarization of the slow wave is linked to the stimulus, this (the depolarization phase) is most probably the electrically excitable phase of the slow wave." This property is also not easily explained by the oscillating electrogenic Na-pump hypothesis unless the assumption is made that the electrogenic Na-pump activity is voltage dependent. This assumption seems unjustifiable since evidence exists that the activity of the electrogenic Na pump in molluscan neurons is independent of the membrane potential (Marmor, 1971).

3. The Spikes:

Here we are faced again with the same problem encountered in the previous discussion of the ionic basis of the resting membrane potential; namely, that very little information is available on the ionic basis of the spikes or "action potentials" in small intestinal smooth muscle. The question has been extensively studied in the taenia coli and uterine smooth muscles (see Kuriyama, 1968 and 1970).

According to the ionic hypothesis (Hodgkin and Huxley, 1952a-d; also Hodgkin, 1958; Huxley, 1959) the action potential in excitable cells has its basis in time- and voltage-dependent changes in ionic conductances of the cell membrane. The regenerative mechanism responsible for the rising phase of the action potential was shown to be due to a voltage-dependent increase in sodium conductance which causes the membrane potential to approach the sodium equilibrium potential (+30 to 50 mV in squid axon). The action potential repolarization was shown to be due to inactivation of the sodium conductance and a delayed increase in potassium conductance.

Although the role of Na ions in the action potential generation in smooth muscle has been a subject of great controversy, nevertheless the basic concept of the ionic theory that the rising phase of the action potential results from an increase in permeability for an ion whose equilibrium potential is in the opposite direction from the resting potential, provides the framework for most of the current studies on the smooth muscle action potential.

In the earlier studies of Holman (1957 and 1958) on the spontaneous spike activity in guinea pig taenia coli smooth muscle,

it was observed that spikes with normal height could be recorded when the bathing solution contained as little as 20 mM Na^+. Below this concentration the spike height was reduced, and at 2 mM Na spontaneous spiking ceased after 30 minutes. However, normal spikes could be recorded for up to 15 minutes in 2 mM NaCl. Axelsson (1961) using the sucrose-gap method observed the effects of Na-deficient solutions on the spontaneous electrical and mechanical activity of the same preparation. His results suggested that both choline and lithium ions can substitute for sodium in carrying the inward current during the rising phase of the action potential. Bülbring and Kuriyama (1963a) studied the effects of sodium and calcium concentrations on the amplitude and rate of rise of the spike. Their results showed that while the amplitude of the spike and overshoot were not dependent on the external Na concentrations between 0 and 137 mM, the rate of rise and fall were dependent. When Na was replaced by Tris spontaneous activity temporarily stopped (probably due to transient hyperpolarization) and then reappeared after 5–10 minutes. Spikes of normal overshoot but with lower rates of rise and fall could be recorded for at least 20 -30 min in the absence of Na. In solutions devoid of both Na^+ and Ca^{++} ions all spontaneous activity ceased and the addition of Ca in the absence of Na^+ restored spontaneous discharges of the action potential for 30 minutes. In Na-free solutions the membrane potential and spike amplitude of taenia coli were found to be a function of the external Ca^{++} concentration. These studies led to the conclusion that the action potential in this tissue was due to Na entry, though the sodium carrier system might be rather limited, but that, in the absence of Na, Ca ions

could carry the inward current (Holman, 1957, 1958; Bülbring and Kuriyama, 1963a). It had also been demonstrated that manganese suppresses spontaneous spike discharge and in higher concentrations also blocks the evoked spikes (Nonomura, Hotta and Ohashi, 1966; Brading, Bülbring and Tomita, 1969; Bülbring and Tomita, 1969a). Tetradotoxin, which blocks nerve action potentials by preventing the increase in sodium conductance, does not abolish intestinal spike discharge (Kuriyama, Osa and Toida, 1966; Nonomura, Hotta and Ohashi, 1966; Bülbring and Tomita, 1967).

More recently, the effects of Na and Ca deficiency have been investigated on the action potentials evoked by electrical stimulation. The results indicated that the contribution of Ca to the action potential may be more important than the contribution of Na (Brading, Bülbring and Tomita, 1969). Kuriyama and Tomita (1970) conducted a double sucrose-gap study on guinea-pig taenia coli electrically evoked action potentials. The evoked spikes were larger in low Na or in Na-free (sucrose substitute) solution than in normal solutions. In Ca-free solutions containing 3-5 mM Mg and 137 mM Na no spikes could be elicited although the membrane potential and membrane resistance were normal. Replacement of Ca by strontium did not abolish the spikes. In another study by Sakamoto (1971) the effects of "Calcium Locke solution" (a solution containing 106 mM $CaCl_2$, 5.6 mM KCl and 5 mM glucose) on electrically evoked action potentials by both the double sucrose-gap and Tomita-type techniques were studied. In this Na-free Ca-Locke solution, the membrane potential hyperpolarized by about 20 mV, the membrane input resistance increased and spikes with overshoot could be

evoked by electrical stimulation. In solutions containing isotonic Sr or Ba instead of Ca ions, spikes could still be evoked. Ba-Locke solution depolarized the membrane, increased its resistance and caused a plateau to occur during spike activity. The effects of Sr-Locke solution on the membrane were similar to those of Ca-Locke solution. Magnesium and cobalt ions were unable to substitute for Ca in supporting the spike generation.

Kumamoto and Horn (1970) carried out a voltage-clamp study on taenia coli muscle using a double sucrose-gap apparatus. They studied the current patterns produced by depolarizing command pulses superimposed on a holding potential of -60 mV. Their study showed that (i) the early transient and steady state currents appear quite similar to those of the squid axon, (ii) tetrodotoxin, in concentrations 100 times those sufficient to completely inhibit the excitability of squid giant axon and skeletal muscle, had no effect on either the early transient or the steady state currents, (iii) the addition of 5×10^{-5} to 5×10^{-4} M $MnCl_2$ to the Krebs solution gradually reduced the early inward current with increasing exposure to Mn, and (iv) in Ca-free solution, there was a gradual reduction and eventual abolition of the early inward current while the steady state current was not affected. From these results the authors concluded that "Considering the available evidence only, the normal mechanism of excitation must be obligate Ca^{2+} dependent but nonspecific in terms of current carriers; in other words, the voltage-dependent gates are not specific for Na^{+}."

It is interesting to note that several observations on the guinea pig taenia coli (and other smooth muscles) resemble those made

on crustacean muscle whose spike is due to Ca entry (Fatt and Ginsberg, 1958; Hagiwara and Naka, 1964; Hagiwara and Nakajima, 1966; Ozeki, Freeman and Grundfest, 1966; Takeda, 1967). For example, the action potential is tetrodotoxin-resistant (Kuriyama, Osa and Toida, 1966; Nonomura, Hotta and Ohashi, 1966; Bülbring and Tomita, 1967). Barium can replace Ca in supporting action potential generation (Hotta and Tsukui, 1968; Bülbring and Tomita, 1968, 1969a); the spike mechanism is sensitive to manganese (Nonomura, Hotta and Ohashi, 1966; Brading, Bülbring and Tomita, 1969).

In summary, it appears certain that in the absence of Na, Ca is able to carry the inward current for the rising phase of the spike. Under normal conditions (normal external Na) two possibilities exist: (i) the inward current is carried by Na ions through gates that are not only voltage- but are also calcium-dependent and tetradotoxin insensitive or (ii) the inward current is actually carried by calcium ions. It appears difficult to distinguish between the two possibilities.

Very little work has been done towards the elucidation of the ionic basis of spikes in small intestinal smooth muscle. Tamai and Prosser (1966) reported that in cat longitudinal smooth muscle cells the spontaneous spikes were eliminated when 60% of the NaCl was replaced by sucrose while they were eliminated only when more than 80% of NaCl was replaced by lithium chloride. Reducing the calcium concentration also reduced the spike amplitude. The spikes were completely abolished when Ca was reduced below 25% of its normal value. The interpretation of these results may be complicated by the possibility that the reported effects on spontaneously discharged spikes may be

related to the effects of the altered ionic environment on the pacemaker mechanisms (slow waves and prepotentials). From this point of view, the isolated circular muscle layer of the small intestine, which is lacking in pacemaker mechanisms, may be a more appropriate preparation for studying the ionic basis of electrically evoked spikes. Nevertheless, Job (1969) studied the ionic fluxes associated with slow waves and spikes in segments of cat jejunum. He claimed that in muscle preparation exhibiting spikes, the maximum influx and efflux of $^{45}Ca^{++}$ were temporally associated with the spikes, and that peaks of $^{42}K^{+}$ efflux, when they occurred, were associated with the calcium efflux peak.

4. The Prepotentials:

The ionic currents responsible for the prepotentials in longitudinal smooth muscle cells of the small intestine (and other gastrointestinal smooth muscle) have never been studied. The problem of analyzing their ionic basis is a difficult one for the following reasons: (i) Few, if any, cells, may show pure pacemaker potentials uncontaminated by electrotonic depolarizations (Holman, 1968) and (ii) in the small intestine, these are small depolarizations (1-5 mV amplitude) which occur after the slow waves have started and before the spike is initiated. Under no condition could the slow waves and spikes be abolished without also abolishing the prepotentials. It is only under such condition that the ionic basis for these potentials can be studied.

PART III

MATERIALS AND METHODS

OBJECTIVES OF THE PRESENT WORK

The aim of the present study was the elucidation of the ionic mechanisms responsible for the slow wave generation in the smooth muscle cells of the longitudinal layer of the small intestine and their relationship to the sodium pump in these cells. The important role played by these slow waves in the myogenic initiation, coordination and regulation of gastrointestinal motor function has been reviewed in the "Literature Review." So also were the studies and hypotheses related to their ionic mechanisms, all of which assign an important role for the sodium pump in their generation. Except for one (Job, 1971), these are based on the speculation that this pump is electrogenic and oscillating (Daniel, 1962, 1965; Job, 1969; Liu, Prosser and Job, 1969; Mills and Taylor, 1971; Weems, Connor and Prosser, 1973). Job's hypothesis (1971) assumes that the slow waves result from oscillations in Na permeability triggered by changes in ATP levels at the cell membrane and that these are the result of oscillations in the activity of the sodium pump. We have, therefore, addressed ourselves to the following questions:

1. What is the nature of the Na-K exchange pump in the smooth muscle cells of the small intestine? More specifically, the question was, is the pump electrogenic? and can its operation lead via rhythmic oscillations in either its rate of activity or its coupling ratio to oscillations in the active membrane current and consequently to oscillations in the membrane potential?

The criterion we used in assessing the electrogenicity of the Na-K exchange pump was that which Ritchie (1971) called "the potassium activated response" which is based on the fact that external potassium (and also internal sodium) is needed for the pump activity. According to this criteron, if the pump is stimulated, e.g., by the readmission of potassium to a tissue previously kept in a potassium-free medium (in K-free medium the pump is inhibited and tissue accumulates Na and loses K), the membrane potential should respond by a hyperpolarization if the pump is electrogenic. This hyperpolarization should share the sensitivities of the sodium pump; i.e., to procedures or agents which are known to inhibit the pump either directly like cardiac glycosides and removal of external K or internal Na or indirectly like metabolic inhibitors and reducing the temperature. It is essential to show that the membrane potential, at least in the early stage of the K-induced hyperpolarization exceeds the potassium equilibrium potential and hence cannot be accounted for by passive ion distribution.

2. What are the effects of procedures or agents which are known to inhibit or stimulate sodium pumping on the slow wave activity in the small intestine? In this connection it was felt worthwhile to study the effect of adrenaline which has been claimed to stimulate electrogenic Na pumping in the longitudinal smooth muscle of the guinea pig colon (Burnstock, 1958).

3. What are the temperature sensitivities of the various parameters of the slow wave (amplitude, rates of rise and fall, frequency...etc)? It was hoped that such a study would differentiate between passive (due to permeability changes) and active (due to electrogenic pumping) ionic currents during the various phases of the slow wave.

4. What is the ionic dependence of intestinal slow waves? This question was investigated by studying the effects of various ionic environments on these waves as well as the effects of agents known to affect the permeability of the membrane to various ions.

In order to explore these questions it was felt mandatory to use the intracellular microelectrode recording technique despite the inherent difficulties in its use particularly in spontaneously contracting preparations consisting of extremely small cells. This technique is the only reliable one to give a true picture of the membrane potential and electrical activity of small intestinal smooth muscle cells.

PART II

OBJECTIVES OF THE PRESENT WORK

MATERIALS AND METHODS

1. Tissue Preparations:

Male white New Zealand rabbits weighing about 5-7 lb were killed by the injection of 10 cc of air into the ear vein. The abdomen was immediately cut open and the ligament of Treitz was located. A segment of the small intestine about 20-25 cm starting about 5 cm below the ligament was removed; the lumen contents were immediately cleaned out by flushing with a modified Tyrode solution previously equilibrated with a 5% CO_2-95% O_2 gas mixture. The segment was then cut into 5 cm pieces and kept in oxygenated Tyrode solution at 4°C.

For the isolation of the longitudinal muscle layers, a 5 cm piece of the jejunum was removed from the Tyrode solution, carefully mounted on a moistened 0.8 cm diameter glass rod. The rod was held horizontally beneath a magnifying lens with field illumination. The mesenteric tissue was removed using very fine forceps, a process which also apparently removed the narrow strip of serosa and longitudinal muscle lying underneath the mesentery. A plane of cleavage could then be seen between the longitudinal and circular layers. The longitudinal layer was then teased away from the underlying circular muscle by gently rubbing it with a smooth tissue paper moistened with normal Tyrode solution. During the whole process of isolation, care was taken not to let the tissue dry out by repeatedly moistening it with normal Tyrode solution.

Using this procedure sheets of longitudinal muscle layer (approximately 1 cm wide and 5 cm long) could be easily isolated.

Figure 3: Section in rabbit jejunal muscle preparation used in this study. Notice the existence of part of the circular muscle layer (bottom of the figure).

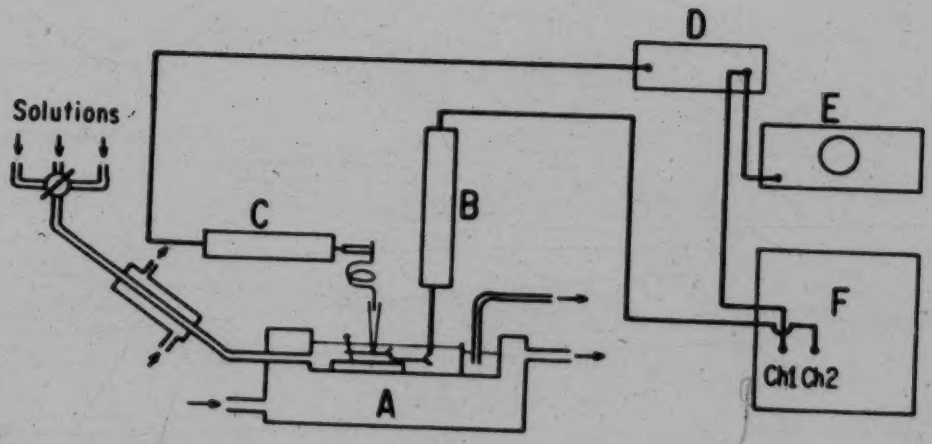

Figure 4: Schematic diagram of apparatus for recording electrical and mechanical activity of rabbit jejunal smooth muscle strips. A, tissue bath surrounded by a constant temperature water jacket; B, transducer for recording mechanical activity; C, the probe of electrometer connected to the microelectrode via a platinum coil; D, electrometer; E, oscilloscope, F; four channel pen recorder (Dynograph). A chlorided Ag wire was immersed in the right-hand end of the tissue bath (reference electrode) and connected to the ground input of the electrometer (not shown in the figure).

Microscopic examination showed that the tissues isolated by this method consisted of the serosa, longitudinal muscle layer, the myenteric plexus and part of the ciruclar muscle layer (Fig. 3). These were kept in normal Tyrode solution at 4°C until further use (usually up to one hour) for either electrophysiological study, sodium enrichment; i.e., storage at 4°C in K-free Tyrode for various time periods, ion content study or for the measurement of the extracellular space.

2. Electrophysiological Studies:

A sheet of the longitudinal muscle, prepared as described above, was spread flat with the serosal layer facing upward and fixed with small pins on 6 x 5 x 0.5 cm block of rubber immersed in oxygenated normal Tyrode. A central piece of the sheet about 4-5 mm wide and 7-10 mm long was cut after fixing a U shaped hook to each of the oral and caudal ends and was then transferred to the recording chamber in which oxygenated normal Tyrode at 37°C was flowing at a rate of 2-3 ml per min. There, one end of the strip was fixed with small pins and the hook at the other end was connected to a mechanical transducer (Fig. 4).

The recording chamber consisted of 6 x 1 x 1 cm perspex bath with an inlet and an outlet for solutions and which had a jacket in which warm water was circulating by a thermostatically adjusted circulating pump. Warm oxygenated solutions flowed through the inlet at a rate of 2 to 3 ml per min and their level in the recording chamber was kept constant by a partition dividing the chamber into a recording chamber proper and an outflow small compartment. Solutions

overflowing into this compartment were removed by a small suction tube. The temperature in the recording chamber was adjusted by adjusting the temperature in the thermostatically controlled circulating pump.

The mechanical activity was recorded via a transducer made of an Euphonics U-15-P cartridge with an N-15-LM stylus (Euphonics Marketing, Chicago) hooked through an appropriately designed coupler to the amplifier of one channel of an R-411 Beckman/Offner Dynograph.

The membrane potential and the electrical activity were recorded by the intracellular floating-microelectrode technique (Woodbury and Brady, 1956). Glass microelectrodes were pulled from Pyrex 9530 melting point glass capillary tubes using a micro-pipette puller (Model M1, Industrial Science Associates, Ridgeway, N.Y.) and were then filled with boiling 50% methanol (v/v) under vacuum and soaked in 3 M KCl overnight. The puller was adjusted to pull electrodes which when filled with 3 M KCl had a resistance between 25 and 50 megohms. After the use of each electrode its tip potential was measured by breaking its tip, reimmersion in solution and observing the magnitude of potential shift. The records from electrode with more than 5 mV tip potential were discarded.

An electrode was suspended from a coil (1.5 to 2 turns, 1 cm in diameter) made of 0.002 inch diameter platinum wire. The coil was connected to the probe of an M4A electrometer (W-P instruments, Hamden, Connecticut) mounted on a Prior micromanipulator. The circuit was completed by connecting a reference electrode(made of a 1.1 mm diameter chlorided silver wire and placed at one end of the recording chamber)to the electrometer. The electrometer output was displayed on both an oscilloscope (Tektronix 502) and another channel of the Beckman/Offner Dynograph.

In the course of this work we adopted the criteria listed by Kao and Nishiyama (1964) for intracellular penetrations. These are,

(a) sharp deflection on penetration to new d-c potential level (in the negative direction),

(b) "cleanliness" of shift on reaching the new d-c level,

(c) persistence of the new d-c level. We decided that the d-c shift from the base-line to the bottom of slow waves should remain constant at least before and after one slow wave (about 4 seconds) and

(d) return to the original base-line after withdrawing the electrode.

A further criterion, recording of large action potentials, was abandoned in our studies since small action potentials were recorded in penetrations which were otherwise perfectly intracellular; these small spikes may have originated in some neighbouring cells and electrotonically conducted from these cells to those from which the recordings were made.

3. Determination of Water, Sodium and Potassium Contents of Tissues

Fresh isolated sheets of the longitudinal muscle layer were equilibrated in oxygenated normal Tyrode solution at 37° C for 1 hour, then weighed and dried in an oven at 110° C for 48 hours and re-weighed. The total tissue water was calculated as the difference between the wet and dry weights. To each tissue sample were added 0.1 ml concentrated nitric acid and 0.05 ml of 35% hydrogen peroxide

solution and the samples were then transferred to a sand bath at 200° C to dry. This treatment was repeated until a whitish residue was obtained. This residue was then dissolved in 25 ml of double distilled (deionized) water and the ion content determined by flame photometry using an EEL flame photometer. Standard curves for sodium and potassium were constructed for each experiment using known but different concentrations of NaCl and KCl. From these standard curves, the ionic content of the sample could be determined by interpolation.

The time course of sodium enrichment and potassium depletion was studied in samples which after equilibration for 1 hour at 37° C were weighed, transferred to K-free Tyrode at 4° C for various time intervals at the end of which they were re-weighed, dried, re-weighed again, digested and their Na^+ and K^+ contents determined as above.

The water and ion contents of recovering Na-rich tissues were also determined in tissue samples which were stored in K-free Tyrode solution at 4° C for 12 hours, transferred to K-free Tyrode at 37° C for 30 min and then transferred to oxygenated normal Tyrode at 37° C for the appropriate time periods at the end of which they were weighed, dried, re-weighed, digested and their Na^+ and K^+ contents determined as above.

4. Estimation of the Extracellular Space:

After equilibration with oxygenated normal Tyrode at 37° C, the tissue samples were transferred on fine hooks, two to a hook, to test tubes containing 5 ml of oxygenated normal Tyrode solution containing a minute concentration of Carboxyl-[^{14}C]-inulin (specific

activity, 1.45-3.65 mC/gm, molecular weight, 5,000-5,500, New England Nuclear Corp.) at 37° C. The tissues were incubated in this medium for varying time periods between 5 minutes and 4 hours. At the end of the incubation period, the tissues were removed, blotted on a filter paper, rinsed rapidly in Tyrode solution and reblotted. This procedure aimed at removing surface adherent radioactivity. The tissues were then placed in pre-weighed scintillation vials and weighed, and were then dissolved using NCS solubilizer (a quaternary ammonium base obtained from Amersham/Searle Corp.) in the amount of 1 ml solubilizer/100 mg tissue weight at 37° C for at least 6 hours.

When the tissue dissolution was complete, Bray's phosphor (Bray, 1960), 10 to 15 ml per vial was added and the total [^{14}C] content measured after the vials had been cooled and dark adapted. Duplicate 1.0 ml portions of media were counted for total [^{14}C] after the addition of 10-15 ml of Bray's phosphor. The total [^{14}C] content was counted using a Picker Nuclear Liquid Scintillation Spectrometer (Liquimat 110) with a counting efficiency for [^{14}C] of about 95%. All vials were counted for a period sufficient to give a less than 1% statistical error of counting.

Correction for the background activity was done by constructing a graph correlating the weight of unlabelled tissue samples to the number of counts arising from their background activity. For each tissue sample used in inulin uptake studies, the background activity was found from this graph and subtracted from the number of counts. For the incubation media, the background activity was determined for each set of experiments as the number of counts in an equal volume of non-labelled medium.

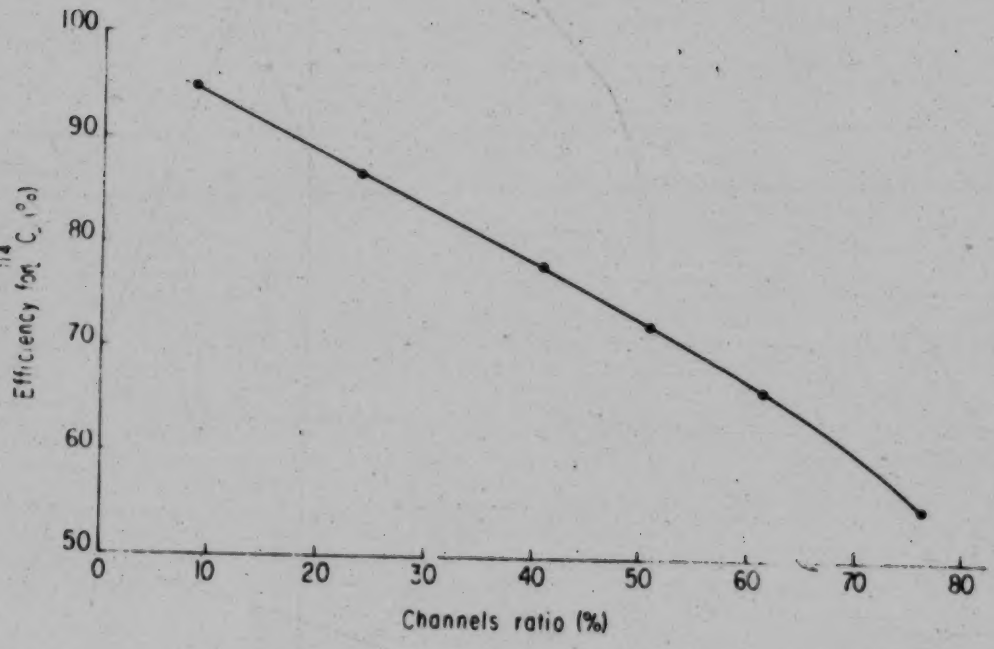

Figure 5: Quench correction curve for [^{14}C] using the channels ratio technique. A series of [^{14}C] quenched standards were counted in two channels; channel A covered the range from 50-850 and channel B from 50-380. These numbers are arbitrary and only represent the energy of the β-particle. Channels ratio was calculated by dividing the cpm in Channel B by the cpm in Channel A.

After subtracting the background activity, correction for the counting efficiency was made by the channels ratio technique (Baille, 1960; Wang and Willis, 1965). This technique is based on counting each sample in two channels of the scintillation spectrometer simultaneously; one of which covered only the first one-third of the spectrum and the second encompassed nearly the whole spectrum. A calibration curve was constructed by counting a series of samples with varying degrees of quenching but containing a known amount of radioactivity and the observed counting efficiency was plotted against the ratio of the counting rates in the two channels (Fig. 5). The experimental samples were counted under identical instrumental conditions, the background activity subtracted, the channels ratio were calculated from the net count rates in the two channels, the counting efficiency for each sample was read from the graph and the disintegrations per min (dpm) was then computed for each sample.

The space occupied by inulin expressed in ml/100 gm wet weight was calculated from the ratio

$$\frac{\text{dpm/100 gm wet weight of tissue}}{\text{dpm/ml incubation medium.}}$$

5. Calculation of Intracellular Ion Concentrations:

The intracellular concentrations of sodium and potassium were calculated using the following equation:

$$\text{Millimoles/liter cell water} = \frac{X_t - X_e V_e}{V_t - V_e}$$

TABLE 1

The Compositions of Solutions

	Normal Tyrode	Na-free (Li)	Na-Def. (Li)	Na-Def. (sucrose)	50% Na (Li)	50% Na (sucrose)	K-free (Na)	Ca-free	Propionate Tyrode	Isethio. Tyrode	Benzenesul. Tyrode	Nitrate Tyrode
NaCl	133.19	—	—	—	56.72	56.72	137.89	133.19	—	—	—	—
LiCl	—	133.19	133.19	—	76.47	—	—	—	—	—	—	—
Sucrose	—	—	—	266.38	—	152.94	—	—	—	—	—	—
Na propionate	—	—	—	—	—	—	—	—	133.19	—	—	—
Na isethionate	—	—	—	—	—	—	—	—	—	133.19	—	—
Na benzenesulphonate	—	—	—	—	—	—	—	—	—	—	133.19	—
Na NO_3	—	—	—	—	—	—	—	—	—	—	—	133.19
KCl	4.70	3.53	4.70	4.70	4.70	4.70	—	4.70	—	—	—	—
K propionate	—	—	—	—	—	—	—	—	4.70	—	—	—
K benzenesulphonate	—	—	—	—	—	—	—	—	—	4.70	4.70	—
K NO_3	—	—	—	—	—	—	—	—	—	—	—	4.70
$CaCl_2$	1.92	1.92	1.92	1.92	1.92	1.92	1.92	—	1.92	1.92	1.92	1.92
$MgSO_4$	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78
NaH_2PO_4	1.17	—	1.17	1.17	1.17	1.17	1.17	1.17	1.17	1.17	1.17	1.17
KH_2PO_4	—	1.17	—	—	—	—	—	—	—	—	—	—
$NaHCO_3$	18.57	—	18.57	18.57	18.57	18.57	18.57	18.57	18.57	18.57	18.57	18.57
$LiHCO_3$	—	18.57	—	—	—	—	—	—	—	—	—	—
Glucose	11.50	11.50	11.50	11.50	11.50	11.50	11.50	11.50	11.50	11.50	11.50	11.50
Na^+	152.93	—	19.74	19.74	76.47	76.47	157.63	152.93	152.93	152.93	152.93	152.93
K^+	$\leq$4.70	4.70	4.70	4.70	4.70	4.70	—	4.70	4.70	4.70	4.70	4.70
Ca^{++}	1.92	1.92	1.92	1.92	1.92	1.92	1.92	—	1.92	1.92	1.92	1.92
Cl^-	141.73	140.56	141.73	0.54	141.73	56.36	141.73	137.89	3.84	3.84	3.84	3.84

where

X_t = total ion (Na or K) content of tissue in mmoles/gm.

X_e = ion concentration in extracellular fluid (Tyrode solution) in mmoles/liter.

V_e = volume of extracellular space (liter/Kgm)

V_t = total tissue water (liter/Kg).

6. Solutions:

The standard solution used was a modified Tyrode solution (Liu, Prosser and Job, 1969). It contained (mM) : NaCl, 133.19; KCl, 4.7; $CaCl_2$, 1.92; $MgSO_4$, 0.78; NaH_2PO_4, 1.17; $NaHCO_3$, 18.57; Glucose, 11.5 and was equilibrated with a gas mixture of 5% CO_2 and 95% O_2, the pH at 37° C was 7.3-7.4.

The composition of solutions in which the concentrations of ions were varied is shown in Table 1.

7. Chemicals:

(a) Carboxyl-[^{14}C]-inulin with a specific activity of 1.45 to 3.65 mC/gm, molecular weight, 5,000 to 5,500 (New England Nuclear Corp.).

(b) 2,4-dinitrophenol (Fisher Scientific Co.).

(c) Ouabain octahydrate (Sigma Chemical Co.).

(d) Epinephrine bitartarate (Nutritional Biochemicals Corp.).

(e) Verapamil hydrochloride; α-isopropyl-α-[N-methyl-N-homoveratryl-γ-aminopropyl]-3,-4-dimethoxyphenyl-acetonitrile; iproveratril, Isoptin. (Knoll AG, Chemische Fabriken, Ludwigshafen am Rhein, West Germany).

8. Statistical Analyses:

The results were expressed as means ± standard errors of the mean. The significance of differences between samples was determined using Student's "t" test. The difference was regarded as significant if p was < 0.05. Simple correlation analysis was also conducted.

The membrane potential values were expressed as the 'true' membrane potential (negative sign was not ignored). Therefore, a depolarization was referred to as an increase and a repolarization as a decrease, in membrane potential. However, a membrane potential that is less negative than normal was referred to as low, and one that is more negative as high, membrane potential.

PART I

LITERATURE REVIEW

CHAPTER 1

THE SPONTANEOUS ELECTRICAL ACTIVITY OF THE SMALL INTESTINE

INTRODUCTION:

The electrical activity of the small intestine in most mammalian species (the only known exception is the guinea pig) consists of two main components: the control activity (known also as the basic electric rhythm, BER) and the response activity composed of smooth muscle action potentials or spikes (see Daniel and Chapman, 1963; Daniel, 1968, 1973; Holman, 1968; Prosser and Bortoff, 1968; Baker, 1969).

The control activity consists of repetitive depolarizations (control potentials, pace-setter potentials or slow waves) of 5-15 mV amplitude, 2 seconds duration which at body temperature occur at a frequency characteristic of the species and the level of intestine from which they are recorded. The response activity consists of one to several spikes associated with the control potentials in some, but not all, cells superimposed on the depolarization phase of the control potential particularly during mechanical activity. Each spike is preceded by a small depolarization or a prepotential (Bortoff, 1961a; Gonella, 1964, 1965; Hukuhara and Fukuda, 1968).

The purpose of this chapter is to describe the spontaneous electrical activity of rabbit jejunal longitudinal muscle strips with particular reference to the configuration of the control potential and its implications regarding the ionic basis and triggering mechanisms of the intestinal control potential.

RESULTS AND DISCUSSION:

The Spontaneous Electrical Activity of Rabbit Jejunum:

The resting membrane potential of the longitudinal smooth muscle cells of rabbit jejunum (defined as the potential at maximum polarization between control potentials) averaged -54.82 ± 0.31 mV (218 penetrations in 60 tissues). The spontaneous electrical activity of these cells consisted of two main components; the control activity and the response activity. The control activity consists of repetitive depolarizations of 17.90 ± 0.19 mV (208 penetrations in 57 tissues) amplitude and 1.97 ± 0.01 sec duration (time from onset of depolarization to end of repolarization) and which recurred at a frequency of 17.55 ± 0.21 per min (Fig. 6A,B,C). These are called the control potentials (also called slow waves or pace-setter potentials) since they are believed to control the excitability of the smooth muscle cells so that response activity (spiking) can occur only during their depolarization phase; the part of the cycle in which excitability is highest.

Some, but not all, cells of the preparations exhibited response activity. This consisted of one to a burst of up to 8 spikes superimposed on the rising phase of the control potential (Fig. 6A,B). Since the permanent records used in the analysis of our data were the pen recordings of the Beckman Dynograph which had a frequency response lower than that needed to faithfully record the response activity (but adequate for recording the control activity), little quantitative significance can be attributed to the values of the spike amplitude or duration measured in this study. The spike amplitude varied from one cell to

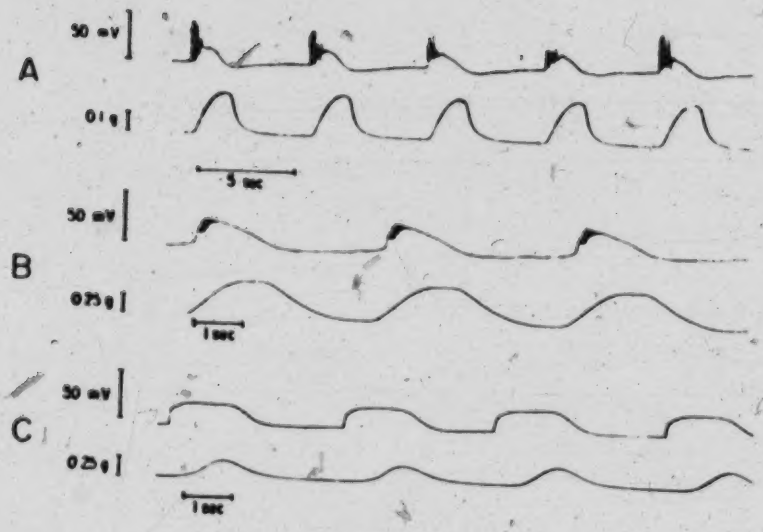

Figure 6: The spontaneous electrical and mechanical activity of longitudinal muscle strips of rabbit jejunum at 37° C. The upper and bottom tracings in each panel show the electrical and mechanical activities, respectively. In this and subsequent figures the top of the voltage calibration lines indicate zero potential. In panels A and B the cells showed intercontrol-potential depolarizations. Notice differences in time scale between panels.

another but never exceeded 35 mV and the spike duration (measured at the inflexion point between the prepotential and the spike) was 15.0 ± 5.3 msec (36 penetrations). The variability of spike amplitude is not likely a reflection of damage to some cells (those which showed low amplitude spikes) upon penetration since (a) the mean resting membrane potential of cells showing low amplitude spikes (less than 10 mV) was not significantly different from that of cells with higher amplitude spikes (-54.4 ± 0.9 mV versus -53.7 ± 0.7 mV respectively) and (b) the resting membrane potential did not significantly decline with time during penetrations (Fig. 6A,B). Cells which exhibited low amplitude response activity might have suffered from a damage to their "spike generating mechanisms" or the response activity recorded from them might have been spreading from neighbouring cells. Each spike was preceded by a small depolarization of up to 4 mV called a prepotential. The prepotential probably directly triggers the spike. Usually each spike was followed by an after-hyperpolarization which sometimes exceeded the resting membrane potential.

Intercontrol-Potential "Diastolic" Depolarization:

In most cells the period between successive control potentials (the intercontrol-potential period) was equipotential; i.e., the potential between the end of a control potential and the onset of the next one remained steady (Fig. 6C). A few cells, however, exhibited a 'diastolic' slow depolarization between successive control potentials which amounted up to 6 mV (intercontrol-potential depolarization) (Fig. 6A,B). Inter-control-potential depolarization was never reported before. Its ionic

basis is unknown. Although its physiological function is not clear, it is interesting to speculate that (a) this depolarization may function as a trigger for the control potential in longitudinal smooth muscle cells and (b) cells with the fastest intercontrol-potential depolarization rate may be the dominant driving oscillators in the preparation. If this was so, then cells with equipotential intercontrol periods may represent the driven cells in the preparation. That this is likely is substantiated by the recent demonstration that control potentials, contrary to earlier belief (Kobayashi, Prosser and Nagai, 1967), are electrically excitable (Mills and Taylor, 1971; Specht and Bortoff, 1972) and that their frequency can be altered by displacement of the membrane potential in either the hyperpolarizing or depolarizing direction (Weems, Conner and Prosser, 1973). Furthermore, such depolarizations were required for the relaxation oscillator model developed by Sarna, Daniel and Kingma (1971) to describe the control activity in the small intestine.

The Configuration of the Control Potential:

The configuration of the control potential could best be studied in cells which showed control, but no response activity (Fig. 6C). In the majority of these cells the control potential appeared as a depolarization phase, a plateau and a repolarization phase. The depolarization time (time from onset of depolarization till maximum depolarization was reached) averaged 155.6 ± 4.0 msec and the mean repolarization time (time from onset to completion of repolarization) was 792.7 ± 6.1 msec (171 penetrations). The rate of rise was generally more variable than the rate of fall of the control potential.

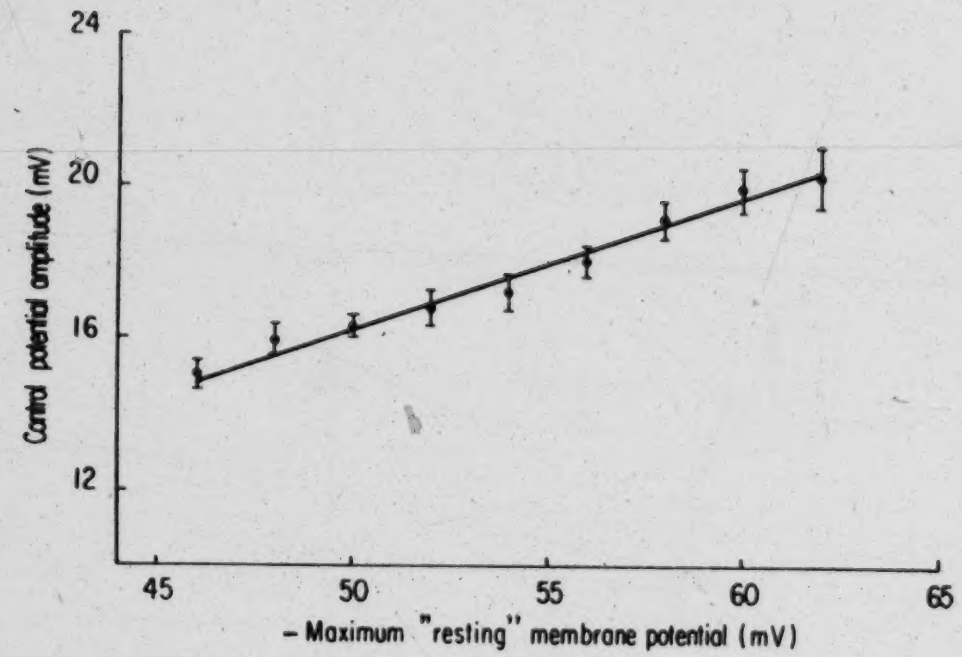

Figure 7: The relationship between the maximum "resting" membrane potential and control potential amplitude. The dots and bars represent experimental means and standard errors respectively. The line represents the regression equation $Y' = 0.34\ X - 0.83$.

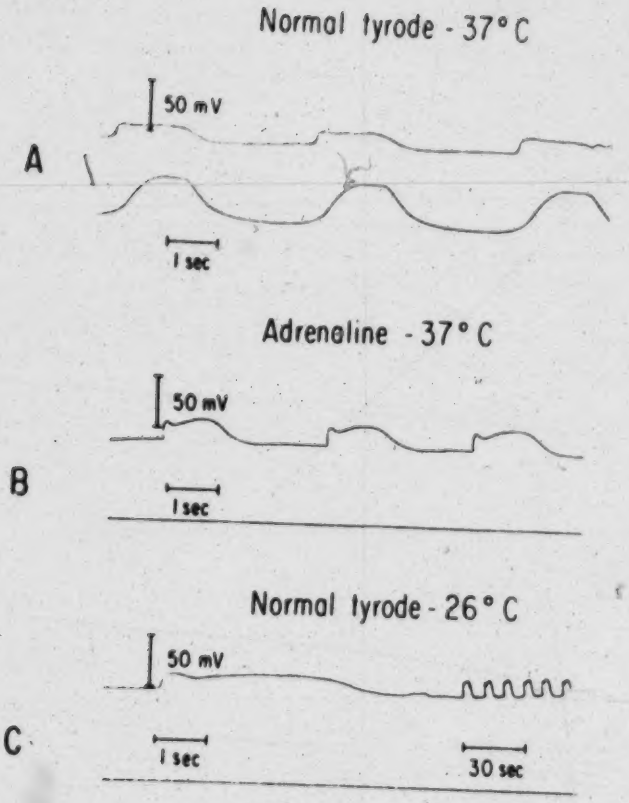

Figure 8: Notching as a genuine feature of control potentials. A, normal Tyrode solution at 37° C, the microelectrode is getting dislodged at the end of the tracing; B, adrenaline (1×10^{-6}M) at 37° C; C, normal Tyrode solution at 26° C. Notice absence of mechanical activity (bottom tracings in each panel) in B and C.

Statistical analysis of the relation between the resting membrane potential and the control potential parameters (amplitude, duration, and depolarization and repolarization times) was conducted. Of all the parameters, only the control potential amplitude showed a significant positive correlation with the resting membrane potential (Fig. 7). The product moment correlation coefficient was +0.542 which was significantly different from zero at the one percent level. Regression analysis showed that the correlation is not significantly different from linearity.

Notching:

In some cells a notch of 1-4 mV appeared persistently early in the plateau phase of the control potentials (100-300 msec after the maximal depolarization was reached). Such notched control potentials generally had rates of rise which, though normal, were in faster side of the normal range (Fig. 8A). This phenomenon was observed, though on rare occasions, by Tamai and Prosser (1966) who attributed it to the electrotonic spread of a spike from a neighbouring cell. To investigate whether notching was an artifact due to either mechanical activity or the electrotonic interaction with a neighbouring spiking cell, the effect of adrenaline on notching was studied. Notching could still be observed in the presence of a concentration of adrenaline (1×10^{-6} M) which was sufficient to stop all spiking and mechanical activity in the preparation and to hyperpolarize the membrane by 6-10 mV (Fig. 8B). Notching was much more common at lower temperatures when spiking and mechanical activity were rare (Fig. 8C). Thus it was concluded that

notching is neither a mechanical artifact nor could it be due to the electrotonic spread of a spike from a neighbouring cell. Notching, then, reflects the operation of two steps in the generation of the control potential. The first step leads to an "initial depolarization" and the second causes a "secondary depolarization." Often the two steps occur sufficiently close in time so that the control potential appears as a depolarization phase, a plateau and a repolarization phase (unnotched control potential). Less frequently, the two steps may be separated in time enough to exhibit a notch on the plateau phase reflecting the turning off of the initial step (repolarizing phase of the notch) before the turning on of the second step becomes sufficient to maintain the initial depolarization.

Notching as a genuine feature of the control potential cannot be accounted for by any of the ionic mechanisms proposed for the control potential generation. These are the oscillating electrogenic sodium pump hypothesis which attributed the control potential to the turning off (depolarization) and on (repolarization) of an electrogenic sodium pump (Daniel, 1962, 1965; Liu, Prosser and Job, 1969; Weems, Conner and Prosser, 1973), and the oscillating sodium permeability hypothesis which suggests that the control potentials are the result of turning on and off of an increase in sodium permeability (Job, 1971). An alternative ionic hypothesis is presented in Part IV, Chapter 3.

CHAPTER 2

EVIDENCE FOR AN ELECTROGENIC SODIUM PUMP IN RABBIT SMALL INTESTINAL SMOOTH MUSCLE AND ITS CONTRIBUTION TO THE MEMBRANE POTENTIAL

INTRODUCTION:

The existence of an electrogenic sodium pump which is capable of providing a direct contribution to the transmembrane potential has been established in a wide variety of tissues including nerve axons, nerve cells, cardiac and skeletal muscle (see Kerkut and York, 1971; Kernan, 1970; Thomas, 1972). Its existence in the smooth muscle cells of the uterus (Daniel, Paton, Taylor and Hodgson, 1970; Daniel, Robinson, Kidwai, Wolowyk, Taylor and Paton, 1971; Taylor, Paton and Daniel, 1969, 1970, 1971) and guinea-pig taenia coli (Casteels, Droogmans and Hendrickx, 1971a, 1973a,b; Tomita and Yamamoto, 1971) has recently been established. Oscillations in the activity of an electrogenic Na pump have been postulated as the basis of the control activity in small intestinal smooth muscle cells (Daniel, 1962, 1965). The control activity in intestinal smooth muscle consists of spontaneous repetitive depolarization-repolarization cycles (control potentials or slow waves) which originate in the longitudinal muscle cells and propagate non-decrementally in the longitudinal axis and electrotonically to the circular muscle layer (see Daniel, 1968, 1973; Holman, 1968; Prosser and Bortoff, 1968). Based on his findings that the control activity in dog's small intestine was sensitive to temperature, hypoxia, metabolic poisons and inhibitors of the Na pump, Daniel (1962, 1965)

suggested that turning-off and on of an electrogenic Na pump may be the cause of the depolarization and repolarization phases of the control potential respectively. Three conditions have to be satisfied if this hypothesis is to be accepted: (1) the sodium pump in the smooth muscle cells of the small intestinal longitudinal muscle has to be electrogenic, (2) the magnitude of contribution of this pump to the membrane potential has to be at least equal to the amplitude of the control potential, and (3) it has to be shown that the pump oscillates spontaneously; i.e., turn off and on so as to provide changes in the transmembrane current that can account for the amplitude, configuration and frequency of the control potentials.

The study reported here was conducted to investigate the electrogenicity of the sodium pump in the longitudinal smooth muscle cells of rabbit jejunal muscle strips. The most widely accepted criterion for illustrating the electrogenicity of the Na pump is to demonstrate that stimulation of this pump (e.g., by admitting K to K-depleted, Na-rich tissues previously kept in a K-free medium) hyperpolarizes the membrane beyond the K equilibrium potential (E_K). The K-induced hyperolarization must be shown to be sensitive to procedures known to inhibit the Na pump; e.g., cardiac glycosides, withdrawal of external K, replacement of internal Na by Li etc. (Richie, 1971; Koketsu, 1971). The data presented here strongly indicate that the Na pump in the longitudinal smooth muscle cells of the rabbit jejunum can be electrogenic.

RESULTS:

As previously reported (Chapter 1--Results and Discussion), the resting membrane potential of the smooth muscle cells of rabbit jejunum longitudinal muscle strips is -54.82 ± 0.31 mV. The spontaneous electrical activity of this preparation consists of repetitive depolarizations of 17.90 ± 0.19 mV (control potentials) recurring at a frequency of 17.55 ± 0.21 per min, and spikes occuring at the depolarization phases of the control potentials in some, but not all, cells.

Time Course of Potassium Depletion and Sodium Enrichment:

Groups of muscle strips (each consisting of two subgroups of 8 samples each) were dissected, immersed in oxygenated normal Tyrode solution at 37° C for one hour, blotted and weighed. One group was taken immediately for ion content analysis and ^{14}C-inulin space measurement, the rest were exposed to K-free Tyrode solution at 4° C for periods of 6, 12, 18 and 24 hours, reweighed and analysed. The intracellular concentrations and equilibrium potentials for Na and K were calculated. These data are presented in Table 2 and Fig. 9. The exposure of the muscle strips to K-free solution at 4° C led to a progressive gain of intracellular Na and loss of intracellular K. These changes were complete after a period of 12 hours of exposure. Thus it was decided to use tissues which had been stored for 12 hours at 4° C in the absence of K throughout this study. These tissues will be referred to as 'Na-rich' tissues.

TABLE 2

Total ion content (mmole/Kg wet wt.), total tissue H_2O (gm/Kg wet wt.) and inulin space (ml/Kg wet wt.) of rabbit jejunal longitudinal muscle strips after exposure to K-free Tyrode solution. From these values the intracellular ionic concentrations (mmole/l. cell water) and the K equilibrium potentials (mV) are calculated

Time in K-free solution at 4°C	$[Na]_{total}$	$[K]_{total}$	$[H_2O]_{total}$	Inulin Space	$[Na]_i$	$[K]_i$	E_K
0 hrs	73.0 ± 0.5 (13)	82.0 ± 2.1 (13)	841.2 ± 8.9 (13)	334.5 ± 15.8 (8)	40.0	161.8	-94.6
6 hrs	133.0 ± 1.2 (8)	11.3 ± 1.3 (8)	876.8 ± 11.8 (8)	324.9 ± 12.3 (8)	148.1	20.5	*
12 hrs	144.4 ± 2.9 (8)	5.1 ± 0.7 (8)	859.7 ± 14.7 (8)	337.2 ± 11.9 (8)	174.5	9.7	*
18 hrs	144.2 ± 4.1 (8)	0.8 ± 0.3 (8)	883.6 ± 12.6 (8)	376.1 ± 16.7 (8)	167.2	1.7	*
24 hrs	151.4 ± 2.9 (8)	0.23 ± 0.1 (8)	867.2 ± 14.3 (8)	350.7 ± 15.4 (8)	186.1	0.4	*

Values expressed as mean ± standard error of the mean.

Numbers in parentheses indicate number of tissues.

* Bathing fluid contained no added K although a low concentration might have been present near the membrane, E_K could not be calculated but must be very large.

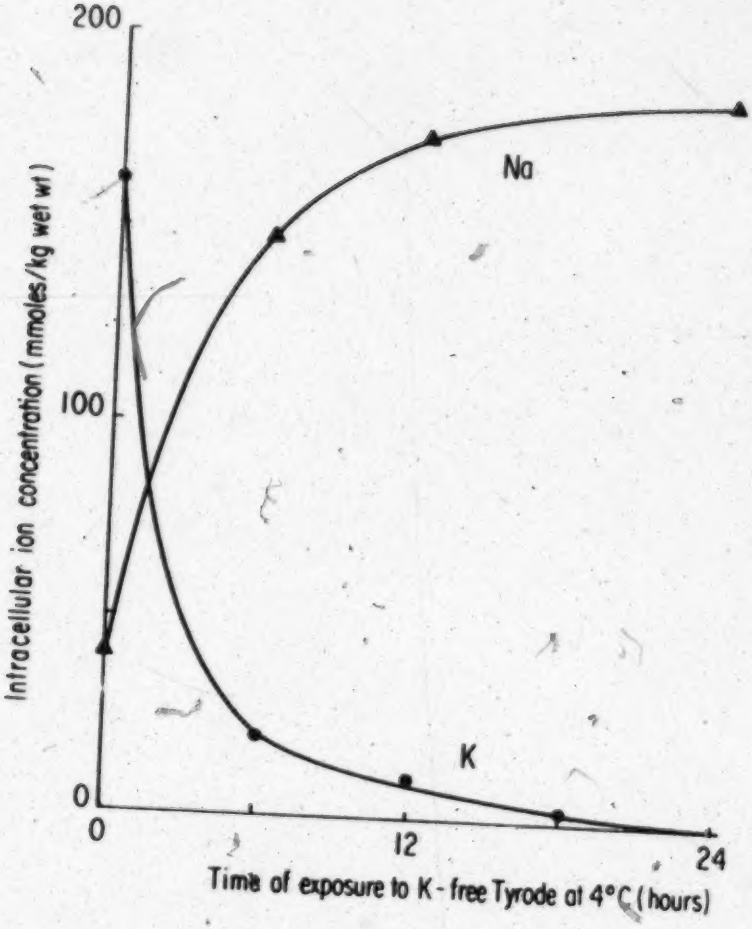

Figure 9: Time course of intracellular Na accumulation and K depletion after exposure of the tissues to K-free Tyrode solution for various times.

Recovery of Membrane Potential of Na-rich Tissues During K Reaccumulation:

Sodium-rich tissues were mounted in the recording chamber in which K-free Tyrode solution at 37° C was flowing. These tissues had a mean membrane potential of -17.54 ± 0.34 mV (92 penetrations in 18 tissues) and showed no spontaneous electrical or mechanical activity. Continued immersion of such Na-rich tissues at 37° C in K-free solution for up to one hour did not change the membrane potential significantly from this value and no recovery of spontaneous activity occurred. However, upon changing solution to normal Tyrode (containing 4.7 mM K) the membrane potential decreased to -74.3 ± 0.7 mV (26 penetrations in 7 tissues) within the first three minutes after the admission of K. This K-induced hyperpolarization persisted for about 20 minutes and then the membrane potential gradually decreased till it stabilized at a mean value of -54.91 ± 0.55 mV (22 penetrations in 7 tissues) 40 to 60 min after K admission (Fig. 10). At this time the spontaneous electrical and mechanical activities had completely recovered. The time course of the recovery of the membrane potential after K admission is illustrated in Fig. 10.

To demonstrate that the K-induced hyperpolarization of Na-rich tissues was higher than the K equilibrium potential, three groups (each consisting of two subgroups of 8 samples each) of Na-rich tissues were rewarmed to 37° C in K-free Tyrode solution for 30 min and then they were exposed to normal Tyrode solution at 37° C for 5, 10 and 15 minutes respectively, weighed and analysed for Na, K and ^{14}C inulin space. The intracellular concentrations and equilibrium potentials for

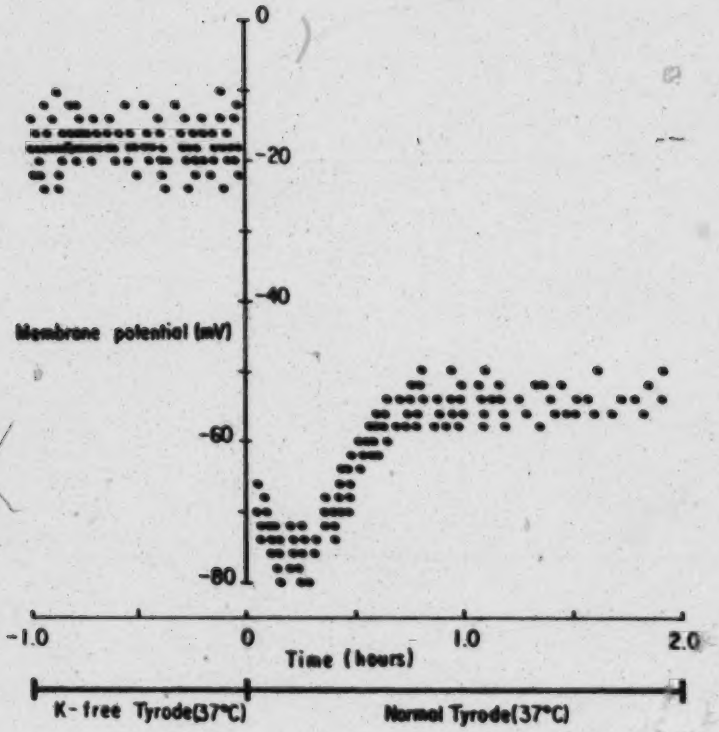

Figure 10: The time course of recovery of membrane potential upon K admission to Na-rich tissues. Seven Na-rich tissues were bathed in K-free Tyrode solution for up to 1 hr. Their membrane potentials are shown to the left of the ordinate. At zero time the solution was changed to normal Tyrode solution (4.7 mM K) and the membrane potential recorded for up to two hr (shown to the right of the ordinate). Each point represents one penetration.

TABLE 3

Total ion content (mmole/Kg wet wt.), total tissue H_2O (gm/Kg wet wt.) and inulin space (ml/Kg wet wt.) after the admission of K to Na-rich tissues. From these values the intracellular ionic concentrations (mmole/l. cell water) and the K equilibrium potential (mV) were calculated

Time after K admission	$[Na]_{total}$	$[K]_{total}$	$[H_2O]_{total}$	Inulin Space	$[Na]_i$	$[K]_i$	E_K
0 min	144.4 ± 2.9 (8)	5.1 ± 0.7 (8)	859.7 ± 14.7 (8)	337.2 ± 11.9 (8)	174.5	9.7	*
5 min	124.4 ± 1.8 (8)	13.1 ± 0.4 (8)	851.3 ± 12.8 (8)	345.1 ± 13.7 (8)	141.5	22.7	-42.1
10 min	121.8 ± 2.3 (8)	21.6 ± 0.5 (8)	860.1 ± 9.6 (8)	340.2 ± 14.1 (8)	134.2	38.5	-56.2
15 min	118.1 ± 2.1 (8)	31.2 ± 0.4 (8)	853.9 ± 11.2 (8)	342.8 ± 15.6 (8)	128.5	57.9	-67.1

Values expressed as mean ± standard error of the mean.

Numbers in parentheses indicate number of tissues.

* Bathing fluid contained no added K although a low concentration may be present near the cell membrane, E_K cannot be calculated but must be very large.

Na and K were calculated. Table 3 shows the equilibrium potentials for potassium of Na-rich tissues after exposure to 4.7 mM K (normal Tyrode) for 5, 10 and 15 minutes. It is clearly obvious that the K-induced hyperpolarization brought the membrane potential to values considerably higher than the K equilibrium potential. It should be emphasized here that there are uncertainties involved in the calculation of the internal ionic concentration which consequently cast doubts on the value of the ionic equilibrium potentials. These arise from the assumptions made in the estimation of the extracellular space (see Goodford, 1968) and that no appreciable binding of ions occurs intracellularly. However, if some of the intracellular K was bound then the calculated E_k would be an overestimate and the discrepancy between this value and the observed membrane potential would be larger. Similarly, if the ^{14}C-inulin space gives an underestimate of the extracellular space, the value of this space would have to be 724 ml/Kg wet wt. to give an intracellular K concentration high enough to give a K equilibrium potential as high as the measured membrane potential during the K-induced hyperpolarization of Na-rich tissues. Such a high value for the extracellular space is highly unlikely.

Effect of Ouabain on the K-Induced Hyperpolarization:

To test whether the K-induced hyperpolarization of Na-rich tissues was the result of stimulation of an electrogenic Na pump, the effect of ouabain was studied. The protocol of the experiments was as follows: Na-rich tissues were mounted in the recording chamber and bathed with K-free solution. After 15-30 minutes the solution was

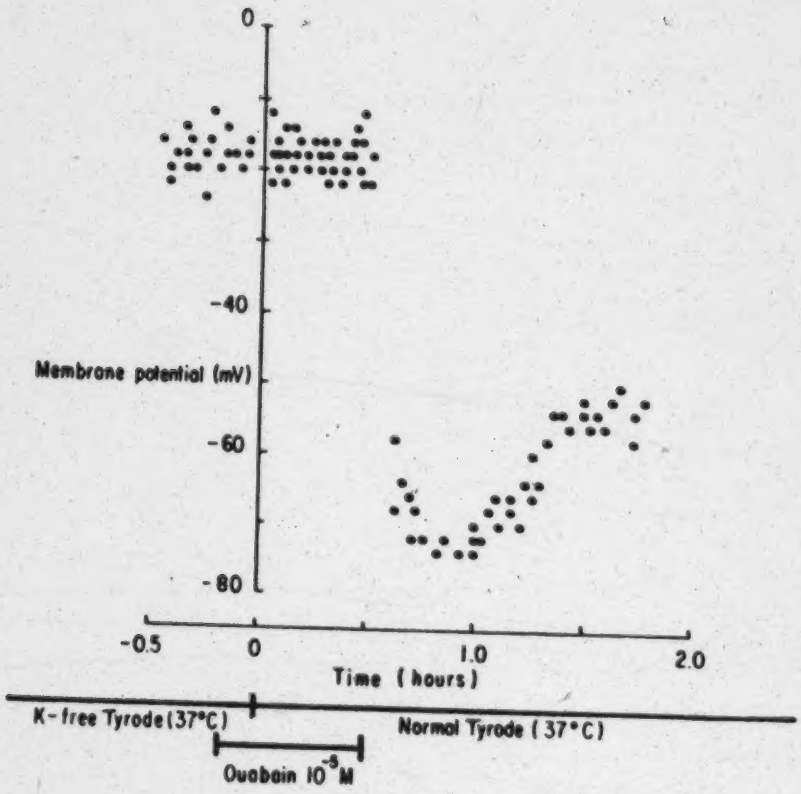

Figure 11: The effect of ouabain (1 x 10^{-5}M) on the recovery of membrane potential of Na-rich tissues. To the left of the ordinate are shown the membrane potentials in K-free Tyrode solution and in K-free solution containing 10^{-5}M ouabain (the last 10 min before zero time). At zero time solution was changed to normal Tyrode solution (4.7 mM K) containing 10^{-5}M ouabain for 30 min after which it was changed again to normal Tyrode solution without ouabain. Each point represents one penetration.

changed to K-free Tyrode solution containing 1 x 10^{-5} M ouabain which was again changed after another 10 minutes to normal Tyrode solution containing the same concentration of ouabain. Finally, after another period of 30 minutes the solution was again changed to normal Tyrode without ouabain. The results of 7 such experiments are illustrated in Fig. 11. The admission of K in the presence of 10^{-5} M ouabain did not produce any significant hyperpolarization in Na-rich tissues. It was only when ouabain was withdrawn that hyperpolarization could occur. Under these circumstances, the decrease in the membrane potential proceeded more slowly than in experiments without ouabain (compare Figs. 10 and 11), presumably reflecting the rate of dissociation of ouabain from membrane binding sites.

Effect of Cooling on the Recovery of Membrane Potential:

In a series of six experiments K was admitted to Na-rich tissues at 6° C. The admission of K at this temperature did not cause any appreciable decrease in the membrane potential of Na-rich smooth muscle cells at least for 30 minutes after K admission (Table 4).

Effect of Admitting K on the Membrane Potential of Li-rich Tissues in the Absence of Na:

Eight muscle strips were made lithium-rich by exposure to K-free, Na-free Tyrode solution, in which all Na has been replaced by Li, at 4° C for 12 hours. When these tissues were rewarmed to 37° C in this solution, they had a mean membrane potential of -15.40 ± 0.76 mV (20 penetrations in 6 tissues). No appreciable hyperpolarization was

TABLE 4

The effect of K admission on the membrane potential (E_m) of Na-rich tissues in which the Na pump had been inhibited by the medium reported in the third column

Tissue	E_m (mV) in K-free solution	Medium changed to	E_m (mV) after changing medium		
			2 - 20 min	20 - 30 min	40 - 60 min
Na-rich	-17.5 ± 0.3 (92)	N.T.	-74.3 ± 0.7 (26)	-68.3 ± 0.9 (15)	-54.9 ± 0.6 (22)
Na-rich	-18.0 ± 0.8 (20)	K-free T.	-16.9 ± 0.5 (30)	-18.2 ± 1.0 (12)	-17.6 ± 1.0 (14)
Na-rich	-17.9 ± 0.6 (22)	N.T. + Ouab.	-17.9 ± 0.6 (22)	-18.4 ± 0.7 (10)	
Na-rich*	-15.9 ± 0.6 (30)	N.T.*	-18.0 ± 0.5 (51)	-16.2 ± 0.7 (23)	
Li-rich	-15.4 0.8 (20)**	Li - T.	-14.9 ± 0.5 (49)	-16.2 ± 0.6 (42)	

Values expressed as means ± standard error of the mean.

Numbers in parentheses refer to the number of penetrations (see text for number of tissues).

* denotes experiments conducted at 6° C.

** Bathing medium was Na-free, K-free (Li) Tyrode solution.

N.T = normal Tyrode.

observed at least for 30 minutes after changing the solution to Na-free (Li-substituted) Tyrode containing 4.7 mM K (Table 4).

Theoretical Analysis of the Contribution of the Electrogenic Na Pump to the Membrane Potential:

In the Appendix to this thesis, a modified Goldman equation was derived so as to account for the contribution of the operation of an electrogenic Na-K exchange pump to the membrane potential

$$E = \frac{RT}{F} \ln \frac{P_K[K]_o + (1 - \alpha + \frac{\alpha}{r}) P_{Na}[Na]_o + P_{Cl}[Cl]_i}{P_K[K]_i + (1 - \alpha + \frac{\alpha}{r}) P_{Na}[Na]_i + P_{Cl}[Cl]_o} \quad (1)$$

where E represents the resting membrane potential, P_K, P_{Na} and P_{Cl} are the membrane permeabilites to K, Na and Cl ions, respectively. $[\ ]_o$ and $[\ ]_i$ refer to extracellular and intracellular ionic concentrations, respectively. R, T and F are the gas constant, absolute temperature and Faraday's constant, respectively. The terms α and r represent the Na-K exchange pump parameters; α is the relative activity coefficient of the pump defined as the ratio between rates of active Na^+ efflux and passive Na^+ influx and r is the coupling ratio of the pump; i.e., the ratio of the rate of active Na efflux to that of active K^+ influx.

This equation has the advantage of being much more general than other equations developed for the same purpose (Mullins and Noda, 1963; Moreton, 1969; Martirosov and Mikhayelyan, 1970; Rapoport, 1970, 1971) in that it does not assume a passive distribution of chloride ions across the membrane or an equality between the passive Na influx and active Na extrusion. It is thus capable of describing any situation provided the net transmembrane current is zero.

TABLE 5

The intracellular and extracellular ionic concentrations of guinea-pig taenia coli, the specific ionic membrane permeabilities and the calculated Goldman Potential

	Casteels, 1969	Brading, 1971
$[K]_o$	5.9 mM	5.9 mM
$[Na]_o$	137.0 mM	136.9 mM
$[Cl]_o$	134.0 mM	133.6 mM
$[K]_i$	164.0 mM	113.98 mM
$[Na]_i$	19.0 mM	3.14 mM
$[Cl]_I$	55.0 mM	25.02 mM
P_K	11×10^{-8} cm/sec	6.71×10^{-8} cm/sec
P_{Na}	1.8×10^{-8} cm/sec	0.066×10^{-8} cm/sec
P_{Cl}	6.7×10^{-8} cm/sec	4.4×10^{-8} cm/sec
Goldman Potential	-37 mV	-57.29 mV

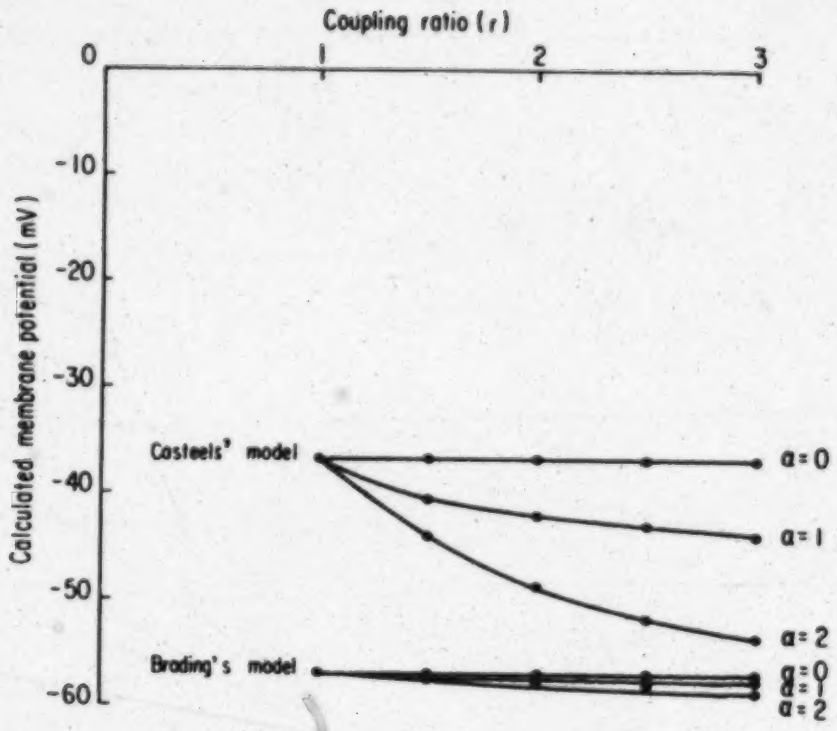

Figure 12: The calculated membrane potentials of smooth muscle cells using the modified Goldman equation at different relative activity coefficients (α) and coupling ratios (r) of the electrogenic Na pump. The upper and lower families of curves represent the calculated potentials from Casteels' (1969) and Brading's (1971) values, respectively of membrane permeabilities and ionic concentrations. In the two sets of curves the horizontal lines at α = zero represent the Goldman diffusion potentials.

Since the permeability of the smooth muscle cell membranes of the longitudinal (or circular) layers of the small intestine were never studied, and hence their values are unknown, we were forced to make our computations using the data obtained from guinea-pig taenia coli. Since there is no general agreement on the values of the membrane permeabilities of taenia coli between workers in this field, the values of the ionic concentrations and permeabilities obtained from two studies (Casteels, 1969; Brading, 1971) were used in the evaluation of the contribution of the electrogenic Na pump (Table 5). The major difference between the two models is that Brading calculated a value of Na permeability that is about 250 times lower than that calculated by Casteels; consequently, Brading's model gives a Goldman potential much closer to the measured membrane potential than that obtained from Casteels' model. To calculate the transmembrane potential using equation (1), one needs values for α and r. It is likely that under physiological conditions the value of α is close to unity otherwise the transmembrane ionic gradients would not be maintained in a steady state. The value of r has been recently calculated by Casteels (1973a) as 1.5 for taenia coli smooth muscle cells. This value is in agreement with those obtained in red blood cells (Garrhan and Glynn, 1967; Sen and Post, 1964; Whittam and Ager, 1965) and suggested for other tissues (see Ritchie, 1971; Kerkut and York, 1971; Thomas, 1972). For each model we constructed a family of curves, each at an assigned value of α (α = 0, 1 or 2); illustrating the relation between the potential calculated using equation (1) and the coupling ratio r. These are shown in Fig. 12. It is obvious that at α = zero, the pump does not

exist and at r = 1 (for any value of α) the pump is electroneutral and in either case the calculated potential is equal to the Goldman potential. The contribution of the electrogenic Na pump is the difference between the Goldman potential; i.e., the horizontal lines in Fig 12 obtained at α = zero and those at other values of α. Comparison of the two families of curves in Fig. 12 shows that at similar values of α and r the contribution of the pump to the membrane potential in Casteels' model is greater than that in Brading's model. This relfects the fact that the lower P_{Na} in Brading's model would imply that a low activity of the pump would be required to balance the passive inward Na leak. It is obvious from Fig. 12 that, at α = 1, neither of the two models is capable of predicting a contribution of the electrogenic Na pump any higher than 9 mV even at the highest coupling ratio. Similarly, at α = 2, the predicted contribution of a pump with a coupling ratio of 3:2 does not exceed 11 mV.

DISCUSSION:

Evidence for the existence of electrogenic Na pumps in a rapidly growing list of tissues has been accumulating in recent years (see Kerkut and York, 1971; Ritchie, 1971; Thomas, 1972). These tissues include nerve cells and axons, skeletal, cardiac and some smooth muscles. The smooth muscles in which an electrogenic Na pump has been demonstrated include the rat uterus (Daniel, Taylor, Paton and Hodgson, 1970; Daniel, Robinson, Kidwai, Wolowyk, Taylor and Paton, 1971; Taylor, Paton and Daniel, 1969, 1970, 1971), guinea-pig taenia coli (Casteels, Droogmans and Hendrickx, 1971a, 1973a,b; Tomita and Yamamoto, 1971) and rabbit detrusor muscle (Paton, 1971). In most tissues, electrogenic Na-pump activity has been detected during conditions of net Na extrusion from tissues containing an elevated intracellular concentration of this ion such as existed in the Na-rich tissues used in the series of experiments presented in this chapter.

In this study of the nature of the Na pump and its ability to contribute directly to the membrane potential in the longitudinal smooth muscle cells of rabbit jejunum we were interested in two fundamental questions: (1) what are the determinants of the resting membrane potential in small intestinal smooth muscle? and (2) what is ionic basis of the intestinal control activity? This activity consists of periodic depolarizations; the control potentials which range from 12-25 mV and arise from a resting membrane potential of -55 mV (Part IV, Chapter 1). Since they were found to be sensitive to hypoxia, temperature, metabolic poisons and inhibitors of the Na pump, Daniel (1962, 1965) postulated that they originate from the oscillatory activity of an

electrogenic Na pump; i.e., repetitive turning off and on of the pump is responsible for the depolarization and repolarization phases of the control potential respectively. The demonstration that the Na pump in this tissue is electrogenic and that the magnitude of its contribution to the membrane potential is at least equal to the magnitude of the control potential is essential for the acceptance of this hypothesis.

The main finding in this study was that a marked rapid hyperpolarization occurred in response to the admission of K (4.7 mM) to Na-rich tissues previously kept in a K-free medium. This K-induced hyperpolarization persisted for about 10-20 minutes and then the membrane potential progressively increased in K containing solution until it reached values comparable to those observed in fresh tissues.

At this point, it is important to consider the possible mechanisms which may have caused the K-induced hyperpolarization in Na-rich tissues. The transmembrane potential can be visualized as the sum of two potentials: (1) a diffusion potential determined by the membrane permeabilities to, and the internal and external concentrations of, Na, K and Cl ions and which can be calculated using Goldman equation (2) and (2) a potential that may be generated by the activity of the Na pump if it operates electrogenically.

$$E = \frac{RT}{F} \ln \frac{P_K[K]_o + P_{Na}[Na]_o + P_{Cl}[Cl]_i}{P_K[K]_i + P_{Na}[Na]_i + P_{Cl}[Cl]_o} \qquad (2)$$

In the resting cell hyperpolarization would be expected from an increase in P_K; a decrease in P_{Na} or P_{Cl} or from increased activity of an electrogenic Na pump or of an inwardly directed electrogenic chloride pump.

In all cases where the hyperpolarization is the result of a change in the permeability of the membrane to one or more ions, the membrane potential approaches but never exceeds the K equilibrium potential E_K;

$$E_K = \frac{RT}{F} \ln \frac{[K]_o}{[K]_i}$$

In Na-rich tissues the observed membrane potential during the period of maximal hyperpolarization after K admission far exceeded the K equilibrium potential calculated from the ionic content and extracellular space measured under the same experimental conditions. These findings are in agreement with the description of the relation between the observed membrane potential and E_K during the K-induced hyperpolarization of Na-rich rat myometrium given by Taylor, Paton and Daniel (1970) and that given by Casteels, Droogmans and Hendrickx (1971a) for the guinea-pig taenia coli. In the latter study, however, the increase in membrane potential in response to K admission to Na-rich tissues initially followed the change in E_K and it was only 5-7 minutes after K admission that the membrane potential became more negative than E_K. In our studies, as well as those on the rat myometrium (Taylor, Paton and Daniel, 1970) the K-induced hyperpolarization occurred much faster than in taenia coli (Casteels, Droogmans and Hendrickx, 1971a) such that the membrane potential was more negative than E_K as early as 2 or 3 min after K admission. This difference might be the result of differences in the experimental procedure or to differences between tissues and/or species.

Having established that the K-induced hyperpolarization of Na-rich tissues cannot be explained by passive permeability changes, since the membrane potential values were more negative than E_K, the next step was to establish that this hyperpolarization shares common characteristics with the Na pump. Since the work of Skou (1957), several workers have shown a close relationship between the properties of active Na transport and those of a (Na + K) activated membrane ATPase (see Skou, 1965; Heinz, 1967; Albers, 1967; Glynn, 1968; Bonting, 1970; Caldwell, 1970 for references). The transport process and the enzyme share common characteristics; both

(1) require ATP as the substrate (enzyme) or the metabolic source of energy (transport process),

(2) are inhibited by cardiac glycosides (e.g., ouabain); the effects of ouabain can be overcome by increasing the external K concentration,

(3) show an absolute requirement for Na and Mg ions on the inside of the membrane. No other ion can replace Na,

(4) require K on the outside of the membrane but other monovalent cations (e.g., Rb, Cs or NH_4) can substitute K,

(5) are located in the cell membrane.

These similarities strongly suggest that the ATPase enzyme is closely linked with the Na pump (Skou, 1965, 1971; Caldwell, 1968, 1970). If the K-induced hyperpolarization was the result of the electrogenic nature of the Na pump in rabbit jejunal smooth muscle,

then one would expect this hyperpolarization to share these common properties of the active Na transport and the (Na + K) activated membrane ATPase. This prediction has been fulfilled in studies on the electrogenic nature of the Na pump in a wide variety of tissues (see Kernan, 1970; Kerkut and York, 1971; Ritchie, 1971; Thomas, 1972 for references). In the present work, we have shown that the K-induced hyperpolarization of Na-rich tissues failed to occur in the absence of K, at low temperature (6° C), in the presence of ouabain (1×10^{-5} M) or when internal and external Na were replaced by Li (no hyperpolarization occurred when K was admitted to Li-rich tissues in the absence of Na). Thus we concluded that, since the K-induced hyperpolarization (a) brought the membrane potential to values more negative than E_K (and thus is not explainable by passive permeability change) and (b) showed characteristics similar to those of the active Na transport and the (Na + K) activated membrane ATPase, the Na pump in the longitudinal smooth muscle cells of rabbit jejunum can be electrogenic and that the K-induced hyperpolarization represents stimulation of the activity of this pump.

The question of the contribution of the activity of the electrogenic Na pump to the membrane potential under normal conditions is still unsettled. However, Thomas in his excellent review (1972) concluded that "... it is now becoming clear that the pump is often, perhaps always, electrogenic, although not often making more than a small contribution to the membrane potential." In smooth muscle cells the calculation of the membrane potential using the Goldman equation (Goldman, 1943; Hodgkin and Katz, 1949) often gives values considerably

more positive than the observed membrane potentials (Casteels, 1969, 1970). Despite the fact that the calculated Goldman potential is subject to all the uncertainties involved in the analytical procedures for, and the assumptions made in, the determination of the extracellular space, ionic contents and ionic permeabilities, the discrepancy has been attributed to the operation of an electrogenic Na pump. It is important here to emphasize the fact that the potential described by the Goldman equation is a diffusion potential determined by the ionic concentration gradients and the transmembrane permeabilities and can describe the membrane potential only if the Na pump was either nonexistent or electroneutral.

Since the demonstration of the electrogenic nature of the Na pump, there had been several attempts to modify the Goldman equation so as to account for the contribution of an electrogenic Na pump (Mullins and Noda, 1963; Moreton, 1969; Martirosov and Mikhayelyan, 1970; Rapoport, 1970, 1971). All these modifications depended upon some assumptions which may be in serious error; e.g., most of them assume a passive distribution of chloride ions across the membrane. Furthermore, they only deal with situations that are restricted to conditions in which the net passive Na influx exactly balances the active Na efflux. For these reasons we derived a modified equation (see Appendix) that does not assume either passive Cl distribution or equality of net passive and active Na fluxes; the only restriction being the assumption, which is also assumed in the Goldman equation, that the net membrane current is equal to zero. We needed this equation to calculate the contribution of the electrogenic Na pump to the membrane potential of

intestinal smooth muscle cells and to evaluate whether the turning off and on of the pump can result in membrane potential oscillations; the amplitude of which is, at least, as big as the amplitude of the intestinal control potential; i.e., about 18 mV. Since the author is not aware of any studies in which the ionic permeabilities and concentrations has been estimated in these cells, the values obtained in two different studies (Casteels, 1969; Brading, 1971) on the guinea-pig taenia coli, where the Na pump is electrogenic, were used. The value of the relative activity coefficient of the pump, α, was assumed as unity, a condition which is necessary for the maintenance of a constant $[Na]_i$ and the value of the coupling ratio, r, was taken as 1.5 (i.e., 3 Na:2 K). Under these conditions neither of the two studies predicted a contribution of the electrogenic Na pump (calculated as the difference between the calculated potential at r = 1.5 and that at r = 1) that is sufficient to contribute the amplitude of the control potential even if the coupling ratio was 3 Na:1 K. It is only if α was as high as 2 and r was as high as 3 that a contribution of 17 mV can be predicted from Casteels' but not from Brading's figures (the main difference between the two studies is that Brading's estimate of P_{Na} is about 250 times lower than that of Casteels). It is very unlikely that these conditions (α = 2 and r = 3) can occur under physiological conditions since there appears to be a general consensus that the coupling ratio in nerve and muscle is normally 1 ATP:3 Na:2 K (see Thomas, 1972). It is also highly unlikely that the pump operation can result in the net extrusion from the cells of twice as much Na as that which passively leaks in as implied if α = 2. So we concluded

that the activity of an electrogenic Na pump in taenia coli cannot contribute more than a few millivolts to the resting membrane potential. It is unfortunate that these calculations could not be made on small intestinal smooth muscle cells which exhibit control activity due to the lack of information on the ionic permeabilities of their membranes and the transmembrane ionic concentration gradients. However, calculations based on any reasonable assumptions about ionic permeabilities, coupling ratio and the ratio of active to passive Na fluxes support the contention of Thomas (1972) that the contribution of the electrogenic Na pump activity to the membrane potential may not exceed a few millivolts.

The work presented in this chapter then provides evidence for the electrogenic nature of the Na pump in the longitudinal smooth muscle cells of the rabbit jejunum but the theoretical treatment, though based on data obtained from guinea-pig taenia coli casts doubts on the ability of this pump to contribute more than a few millivolts to the resting membrane potential. Such a small contribution is inconsistent with the hypothesis that the electrical control potentials (the amplitude of which is 18 mV on the average) result from the turning off and on of the electrogenic Na pump.

CHAPTER 3

THE IONIC MECHANISMS OF INTESTINAL ELECTRICAL CONTROL ACTIVITY

INTRODUCTION:

The electrophysiological basis of the myogenic control of the motor function of the small intestine in most species including man is the occurrence in the smooth muscle cells of the longitudinal muscle coat of spontaneous repetitive membrane potential depolarizations, known as the control potentials, slow waves or pace-setter potentials. This activity, which is collectively referred to as the control activity or basic electrical rhythm (BER), functions to periodically depolarize the smooth muscle cell membranes so that response activity (spike potentials) can be initiated only during the depolarization phases of the control potentials (see Daniel and Chapman, 1963; Daniel, 1968, 1969, 1973; Holman, 1968; Prosser and Bortoff, 1968; Baker, 1969). The control activity is myogenic; it originates in the longitudinal muscle layer (Daniel, Honour and Bogoch, 1960; Bortoff, 1961a,b; Kobayashi, Nagai and Prosser, 1966) and spreads electrotonically to the circular muscle layer (Bortoff, 1965; Bortoff and Sachs, 1970; Kobayashi, Nagai and Prosser, 1966).

Studies on the ionic mechanisms of the intestinal control potential led to the development of three hypotheses. The first is the oscillating electrogenic Na-pump hypothesis originally proposed by Daniel (1962, 1965) and presently supported by the voltage clamp study

of Weems, Conner and Prosser (1973). This hypothesis suggests that the control potential results from turning off (depolarization) and on (repolarization) of an electrogenic Na pump. We have shown that the Na pump in the longitudinal smooth muscle cells of rabbit jejunum can be electrogenic (Part IV, Chapter 2). The second hypothesis (Job, 1969) attributes the control potential depolarization to an increase in Na permeability (P_{Na}) and repolarization to increased electrogenic Na pumping. The third hypothesis is the oscillating Na permeability hypothesis (Job, 1971). According to this hypothesis a buildup in ATP concentration at the membrane "turns on" an increase in P_{Na} which leads to the depolarization phase of the control potential and increases the inward leak of Na ions. The increase in intracellular Na coincident with a high level of ATP stimulates the Na pump. The pump depletes ATP to subthreshold values again, the increase in P_{Na} is turned off and the membrane repolarizes.

In our studies on the configuration of the intracellularly recorded control potential (Part IV , Chapter 1) we observed that in the majority of cells, the control potential consisted of a depolarization phase, a plateau and a repolarization phase. Some cells however, exhibited control potentials which persistently had a small "notch" of 1 to 4 mV early in their plateau phases. The possibility that notching may be a mechanical or an electrical artifact has been excluded. We concluded that notching may reflect the operation of two processes in the control potential generation and that it cannot be accounted for by either the oscillating electrogenic Na-pump hypothesis or the oscillating P_{Na} hypothesis, both of which attribute the control potential to a single "on and off" event.

The work presented in this chapter was conducted to further investigate the ionic mechanisms responsible for the control potential generation.

RESULTS:

The Relation of the Control Activity to the Electrogenic Sodium Pump:

We have presented evidence that the sodium pump in the longitudinal smooth muscle cells of rabbit jejunum can be electrogenic (Part IV, Chapter 2). To establish the relationship between intestinal control potentials and the activity of this pump we studied the effects of inhibition and stimulation of the pump on the electrical activity of this tissue.

Inhibition of the Na pump by 1×10^{-6}M ouabain (Fig. 13), withdrawal of external K (Fig. 14), replacement of Na by Li (Fig. 15), 1×10^{-4}M 2,4-dinitrophenol (DNP), or by cooling below 18° C reversibly abolished the control activity and depolarized the cell membrane. The magnitude of the membrane depolarization varied with time and the type of treatment. The most significant value of the membrane potential under conditions of Na-pump inhibition is that at which the disappearance of the control potentials was first observed. Table 6 summarizes this value immediately after the control activity had been abolished by various treatments that caused inhibition of the Na pump and 40 min after the onset of these treatments. Although all these treatments depolarized the smooth muscle cell membrane, it is obvious that, at

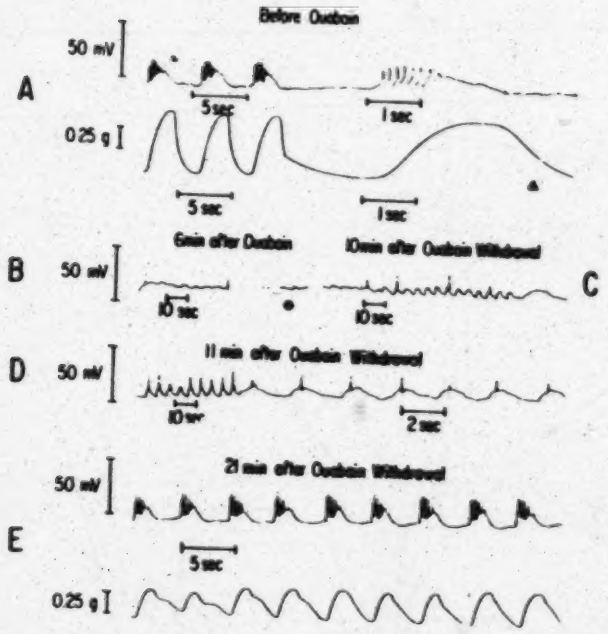

Figure 13: The effect of ouabain (1×10^{-6}M) on electrical control activity. A, normal Tyrode solution changed at ▲ to ouabain-Tyrode solution; B, 6 min after ouabain, solution changed to normal Tyrode at ●; C, D and E, 10, 11 and 21 min after normal Tyrode solution. B, C and D are parts of a continuous record from the same cell. Tissue was mechanically quiescent in B, C and D.

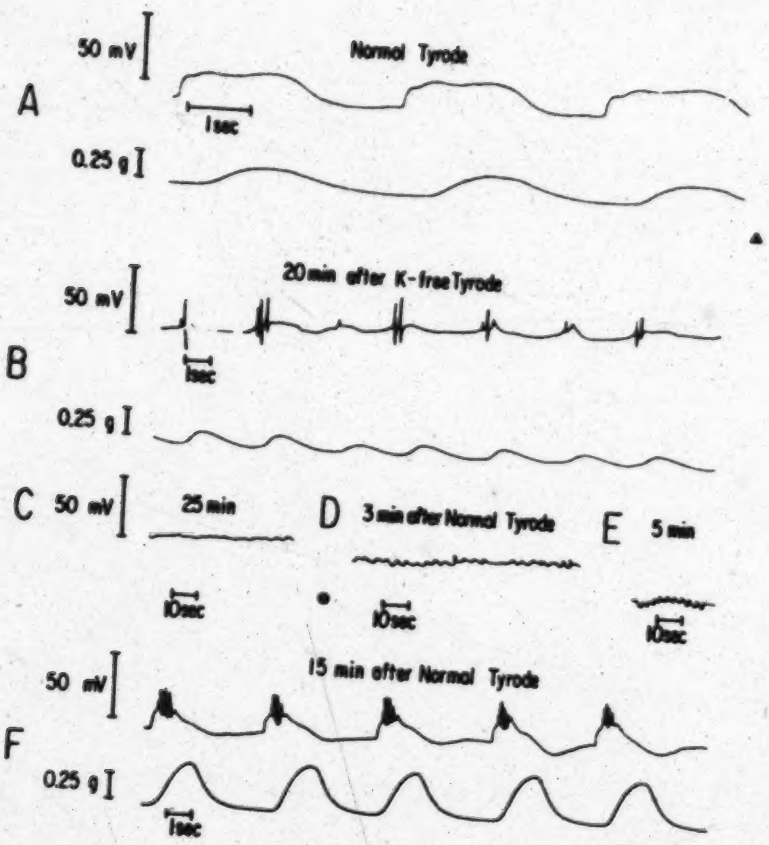

Figure 14: The effect of K withdrawal on the electrical control activity. A, normal Tyrode Solution; B, and C, 20 and 25 min after changing solution to K-free Tyrode; D, E and F, 3,5 and 15 min after changing solution back to normal Tyrode. C and D are parts of a continuous record from the same cell. Mechanical records were excluded from C, D and E. Notice intercontrol-potential depolarizations in F.

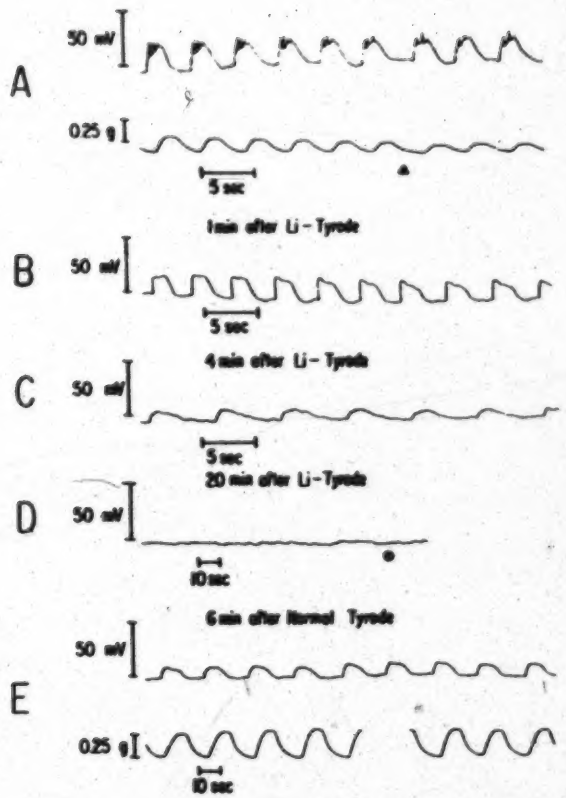

Figure 15: The effect of Na-free (Li) Tyrode solution on the electrical control activity. A, normal Tyrode solution changed to Na-free (Li) Tyrode solution at point marked ▲; B, C and D, 1, 4 and 20 min after Li-Tyrode, solution changed back to normal Tyrode at ●, E, 6 min after normal Tyrode solution. Mechanical tracings were excluded from B, C and D since tissue was mechanically quiescent.

TABLE 6

Membrane potentials of rabbit jejunal smooth muscle cells at the time the electrical control activity first disappeared, and 40 min, following Na-pump inhibition

Na-pump inhibitor	Membrane Potential (mV) at	
	time control potentials ceased	40 min after pump inhibition
Ouabain (1×10^{-6}M)	-45.3 ± 0.8 (19,8)	-38.9 ± 0.5 (25,7)
K-free Tyrode	-49.5 ± 0.6 (13,7)	-59.8 ± 0.5 (9,7)
Na-free (Li) Tyrode	-52.0 ± 0.6 (22,7)	-49.1 ± 0.5 (29,6)
DNP (1×10^{-4}M)	-47.1 ± 0.7 (14,4)	-39.8 ± 0.6 (17,4)
Cooling (15-18° C)	-49.2 ± 0.7 (20,4)	-46.1 ± 0.8 (19,4)

Results expressed as mean ± standard error of the mean.

Numbers in parentheses indicate numbers of penetrations and number of tissues used, respectively.

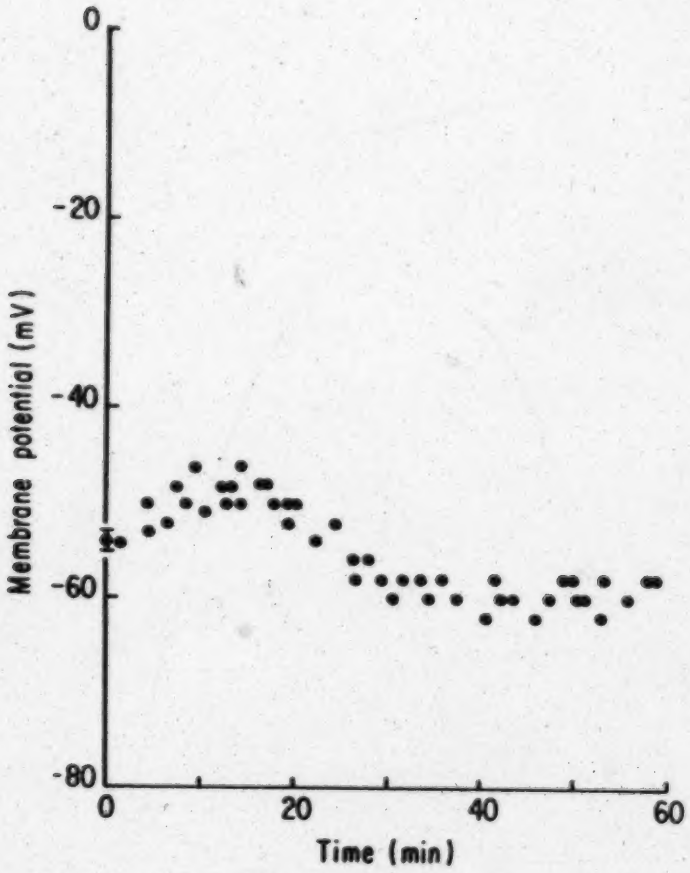

Figure 16: The time course of changes in the membrane potential induced by K withdrawal. At time zero, solution was changed from normal Tyrode to K-free Tyrode. Notice initial increase (depolarization) followed by a decrease (hyperpolarization) in the membrane potential. Each point represents one penetration.

the time the control activity ceased, none of them brought the membrane potential to a value close to the peak depolarization of the control potential (which is -37 mV). This would be expected if the control potential depolarization was due to turning off of the electrogenic Na pump and if the various procedures inhibited the electrogenic Na pump without affecting the membrane permeabilities. The membrane potentials at this time were about 8 to 15 mV more negative than the potential level at the peak depolarization of the control potential. With the exception of K withdrawal, all treatments led to a progressive depolarization, though to different extents as shown from the value of the membrane potentials after 40 min of pump inhibition.

The effect of K withdrawal on the membrane potential was biphasic. There was an initial depolarization of about 5 mV that accompanied the cessation of the electrical control activity during the first 20 min after K omission. After this initial period the membrane gradually repolarized over the period of the next 20-40 min when it reached a value about 6 mV higher than that of normal tissues (Fig. 16). Similar biphasic effects were observed in response to K withdrawal in the guinea-pig taenia coli smooth muscle cells by both Casteels, Droogmans and Hendrickx (1971b) and Tomita and Yamamoto (1971). These authors attributed the initial depolarization to inhibition of the electrogenic Na pump and the secondary hyperpolarization to an increase in the K permeability (P_K).

Recovery of the membrane potential and electrical control activity occurred within 5 to 10 min upon restoring normal Tyrode solution to the bath after any of the above procedures. In the K withdrawal

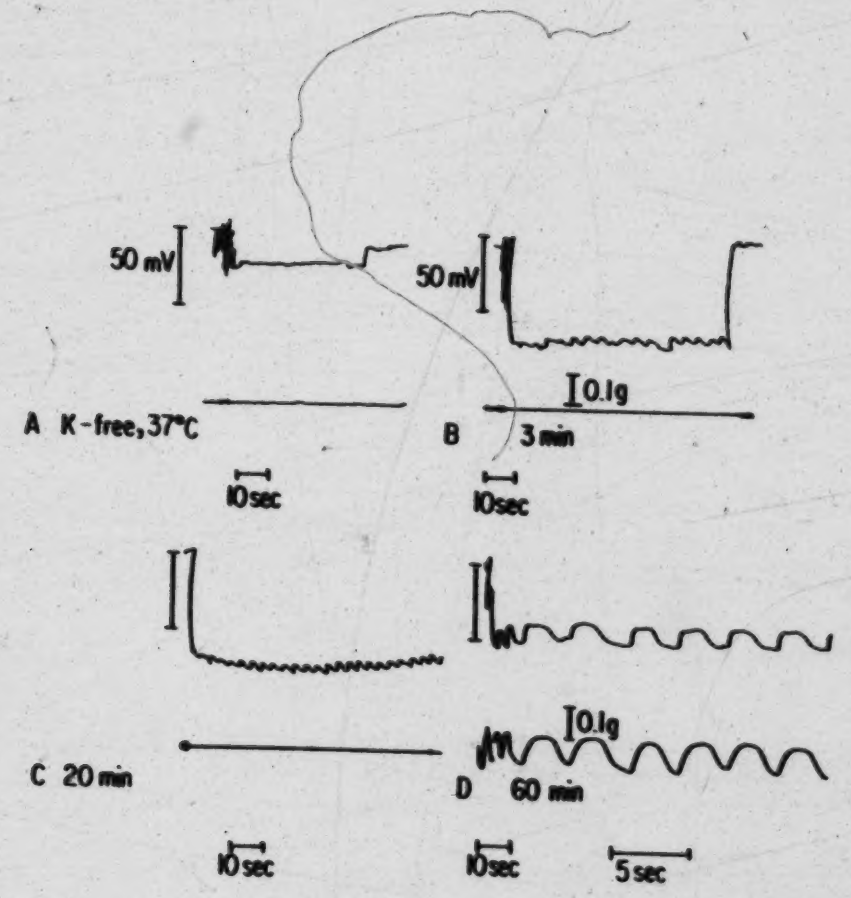

Figure 17: Recovery of the membrane potential and electrical and mechanical activity of Na-rich tissues upon K admission. A, Na-rich tissue in K-free Tyrode solution at 37° C; B,C and D, 3, 20 and 60 min after changing solution to normal Tyrode solution (4.7 mM K). Top and bottom tracings in each panel show electrical and mechanical activities, respectively.

experiments, the readmission of K, particularly after long (30 min or longer) exposure to K-free solution, caused a rapid hyperpolarization and the appearance of small amplitude control potentials (Fig. 14). The membrane potential and the size of the control potentials gradually increased to reach values close to those observed before K withdrawal in 10-15 min.

Stimulation of the electrogenic Na-pump activity was achieved by allowing tissues to accumulate Na intracellulary and to lose their intracellular K (by storing them at 4° C in K-free Tyrode solution for 12 hr as described in Part IV, Chapter 2), rewarming them to 37° C in the absence of K and then admitting K. The admission of K caused an immediate decrease in the membrane potential to about -75 to -80 mV and the appearance of control potentials of very small amplitude (3-8 mV) at an average frequency of 18.13 ± 0.24 per min (23 penetrations in 7 tissues) as shown in Fig. 17B and C. Although quantitation of the rates of depolarization and repolarization of these small control potentials was difficult, it appeared that the rate of depolarization was somewhat slower than, while the rate of repolarization was similar to, that of the control potentials recorded from normal tissues. In the penetrations where they could be measured (5 min after K admission), the depolarization time (time from onset to peak depolarization) was 234.3 ± 20.3 msec and the repolarization time (time from onset to completion of repolarization) was 800.0 ± 19.5 msec (normal values were 155.6 ± 4.1 and 792.7 ± 6.1 msec respectively). The hyperpolarization persisted for about 10 to 20 min after which the membrane potential progressively decreased and the amplitudes of the control potentials

and their rates of rise increased and eventually reached values similar to those observed in normal tissues 45 to 60 min after K admission.

The Temperature Sensitivity of the Electrical Control Activity:

The effects of temperature on the membrane potential and electrical control activity were studied by gradual cooling and rewarming of tissues. The frequency and duration of control potentials exhibited higher temperature sensitivity than did the maximum resting membrane potential or the control potential amplitude. Figure 18 shows parts of a continuous penetration in the same cell while the temperature gradually rose from 24 to 34° C. To facilitate calculation of the temperature coefficients (Q_{10}) for the parameters of the electrical control activity, our studies were done mainly at 37 and 27° C. The frequency of the control potentials progressively increased from 6.68 ± 0.08 (37 penetrations in 8 tissues) at 27° C to 17.55 ± 0.21 per min at 37° C (Q_{10} = 2.61). The maximum resting membrane potential exhibited a slight but significant increase upon cooling; it increased from a value at 37° C of -54.82 ± 0.31 (218 penetrations in 60 tissues) to -52.12 ± 0.39 mV (50 penetrations in 9 tissues) at 27° C. Cooling down to this temperature had no significant effect on the control potential amplitude. Cooling also reduced the frequency of the response activity (spiking) associated with the control potentials. Consequently, the force of the rhythmic contractions progressively decreased and the tight 1:1 coupling of control potential frequency and contraction frequency was lost (occasionally control potentials were not accompanied by contractions) as shown in Fig. 18A and B. At sufficiently low temperatures (about 25° C) the contractile activity was sometimes completely suppressed.

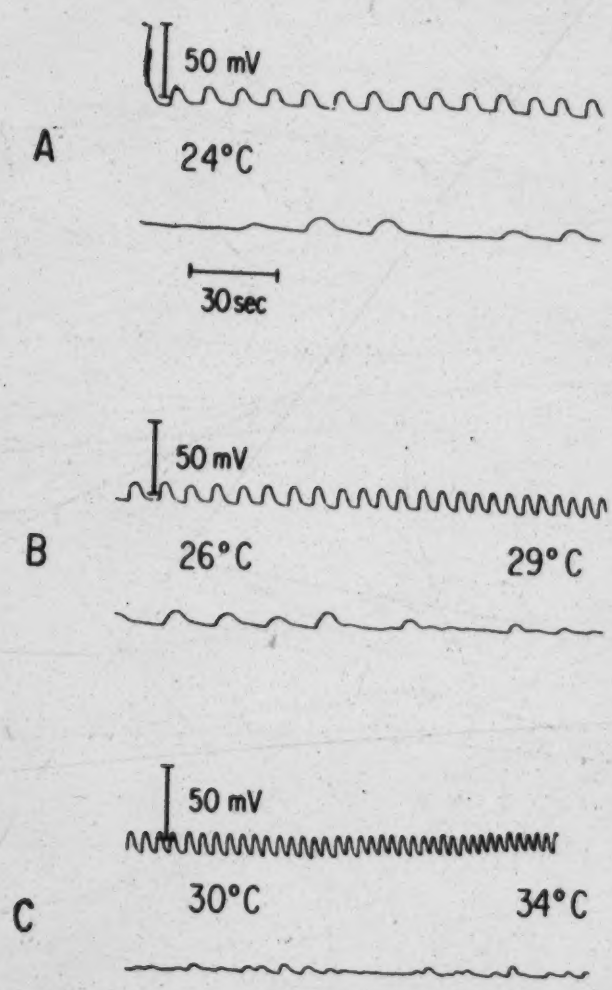

Figure 18: The effect of rewarming on the electrical control activity. Tissue had been cooled to 24° C for a few minutes by switching off the warm water. A, 24° C. Warm water was switched on after A; B, 26-29° C; C, 29-34° C. A, B and C are a continuous record from the same cell. Top and bottom tracings represent electrical and mechanical activities, respectively.

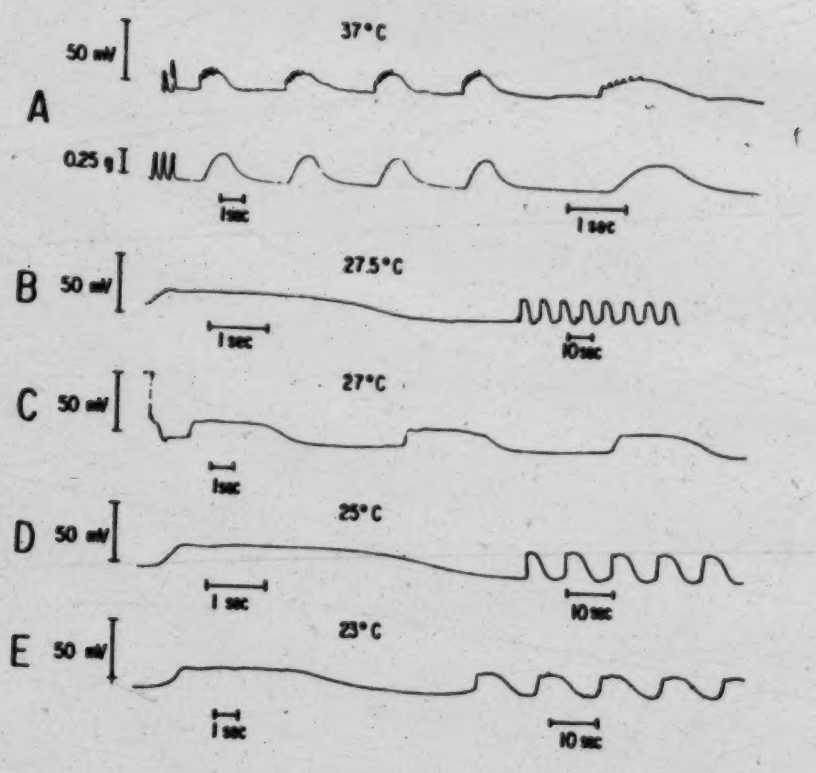

Figure 19: The temperature sensitivity of the electrical control activity. A, 37° C; B, 27.5° C; C, 27° C; D, 25° C and E, 23° C. Notice differences in time scale; first parts of A, C and D were recorded at a paper speed of 10 mm per sec and last part of A, B and E at 25 mm per sec. Control potentials in B, C, D and E exhibited a notch early in their plateau phases.

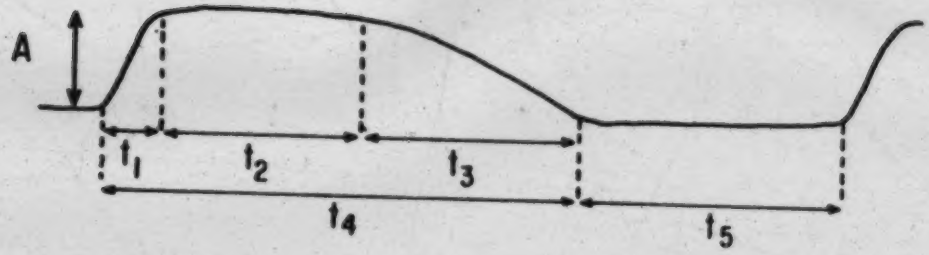

Figure 20: The parameters of the un-notched control potential used in the analysis of its temperature dependence.

TABLE 7

The temperature dependence of un-notched control potentials

	37° C	27° C	Q_{10}*
A (mV)	18.0 ± 0.2 (176)	17.3 ± 0.3 (20)	1.04
t_1 (msec)	159.1 ± 4.6 (139)	240.0 ± 11.1 (19)	1.52
t_2 (msec)	1000.9 ± 5.9 (139)	2540.1 ± 34.1 (20)	2.54
t_3 (msec)	794.5 ± 7.0 (139)	2180.2 ± 30.3 (20)	2.75
t_4 (msec)	1972.0 ± 12.0 (166)	5090.3 ± 31.1 (20)	2.58
t_5 (msec)	1578.8 ± 12.7 (166)	3885.4 ± 20.0 (20)	2.46

Values expressed as means ± standard error of the mean.

Numbers in parentheses indicate number of penetrations.

*Q_{10} values were calculated from values at 37° C and 27° C.

The effects of cooling on the control potential configuration were most interesting and are illustrated in Fig. 19. As the temperature was lowered from 37° C to 23° C notching of the control potentials, which we observed in some cells at 37° C (Part IV, Chapter 1), appeared much more commonly (Fig. 19). We have suggested (Chapter 1) that it is likely that two separate processes occurring in sequence are responsible for the control potential: the first of which causes an "initial depolarization" while the second leads to a "secondary depolarization" and that when the two processes are separated enough in time, notching can occur. The finding that upon cooling more cells exhibited notched control potentials suggested to us that the two processes responsible for these potential oscillations may have different temperature sensitivities which allow their temporal separation to increase as the temperature was reduced. Thus the temperature coefficients $Q_{10\ (37\text{-}27°\ C)}$ for the various phases of the control potential were calculated separately for the notched and un-notched configurations.

In the un-notched control potential, the time from onset to completion of depolarization (t_1) was taken as an indication of the rate of depolarization and the time from completion of depolarization to the onset of repolarization (t_2) as the plateau duration and that from onset to completion of repolarization (t_3) as a measure of the repolarization rate. The times t_4 and t_5 (the times from the onset of depolarization to end of repolarization; i.e., control potential duration and from the completion of repolarization to onset of the depolarization of the next control potential; i.e., intercontrol-potential period, respectively, were also calculated at different temperatures (Fig. 20). The Q_{10} for

the control potential amplitude (A) was also computed. The results of these calculations are shown in Table 7. It should be pointed out here that it was often difficult to decide exactly when each phase started or ended. Nevertheless, this analysis reflects obviously large differences in the sensitivity of these phases to temperature. The amplitude (A) as well as the depolarization time (t_1) of the control potential exhibited the least temperature sensitivities (Q_{10s} being 1.04 and 1.52, respectively). On the other hand, the duration of the plateau phase (t_2) and the repolarization time (t_3) of the control potential showed a high temperature sensitivity (Q_{10s} were 2.54 and 2.75, respectively). Consequently, the high temperature coefficient (Q_{10} = 2.58) for the control potential duration (t_4) cannot be attributed to the sensitivity of the depolarization phase to cooling, but it is indeed due to the delay in the onset and rate of the repolarization phase. The frequency of the control potentials had a Q_{10} of 2.61. The drastic decrease in the control potential frequency is not solely due to the increase in the control potential duration (t_4) but is also partly due to prolongation of the intercontrol-potential period (t_5) which exhibited a Q_{10} of 2.46.

The analysis of the temperature sensitivity of the notched control potential so as to reveal possible differences between the initial and secondary depolarization was achieved by dividing the notched control potential in the manner shown in Fig. 21. To obtain some information on the sensitivity of the process responsible for the initial depolarization, the effects of temperature on its amplitude (A_{1n}) and the time from the onset to completion of the initial depolarization (t_{1n}) were

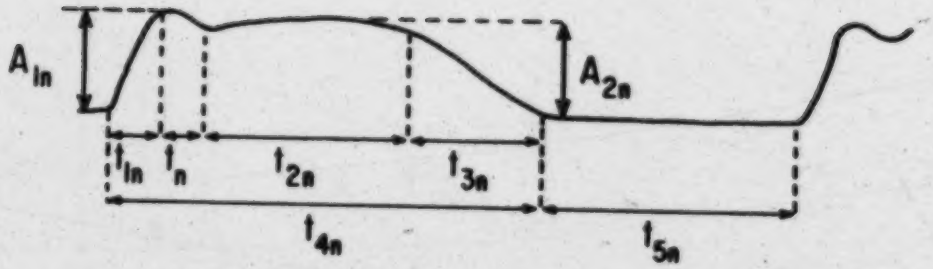

Figure 21: The parameters of the notched control potential used in the analysis of its temperature dependence.

TABLE 8

The temperature dependence of notched control potentials

	37° C	27° C	Q_{10}*
A_{1n} (mv)	17.3 ± 0.3 (32)	17.3 ± 0.3 (30)	1.00
A_{2n} (mv)	16.8 ± 0.3 (32)	16.9 ± 0.3 (30)	1.00
t_{1n} (msec)	140.0 ± 8.4 (32)	219.2 ± 12.0 (29)	1.56
t_n (msec)	166.3 ± 8.1 (32)	277.0 ± 8.0 (30)	1.65
t_{2n} (msec)	871.3 ± 10.4 (32)	2402.9 ± 34.2 (30)	2.76
t_{3n} (msec)	785.0 ± 12.9 (32)	2183.2 ± 28.1 (30)	2.78
t_{4n} (msec)	1960.0 ± 19.0 (32)	5093.1 ± 32.2 (30)	2.60
t_{5n} (msec)	1537.0 ± 15.0 (32)	3900.2 ± 24.4 (30)	2.54

Values expressed as means ± standard error of the mean.

Numbers in parentheses indicate numbers of penetrations

*Q_{10} values were calculated from values at 37° C and 27° C.

analysed although it is conceivable that the second process responsible for the secondary depolarization may also provide some contribution to the initial depolarization. Similar information regarding the secondary depolarization was obtained by analysing the temperature dependence of its amplitude (A_{2n}) as well as that of the following time intervals (Fig. 21):

1. The interval between the peak of the initial depolarization and the bottom of the notch (t_n) .
2. The interval between the bottom of the notch and onset of repolarization (t_{2n}) which was used as a measure of the time during which the second process was fully on (mainly responsible for the plateau of un-notched control potentials).
3. The period between the onset and completion of repolarization (t_{3n}) which gives some estimate of the rate of turning off of the second process.
4. The control potential duration (t_{4n}) measured as the period from the onset of the initial depolarization to the completion of repolarization.
5. The intercontrol-potential period (t_{5n}) measured as the time from the complete repolarization of a control potential to the onset of the initial depolarization of the following control potential.

The results of this analysis are summarized in Table 8. It is obvious that, in the temperature range between 37° C and 27° C, there was no observable temperature dependence of the amplitudes of both the initial (A_{1n}) and secondary (A_{2n}) depolarization phases (Q_{10} = 1.00

in both cases) while an important difference between the rates of both phases could be revealed. The depolarization time of the initial depolarization (t_{1n}) had a low Q_{10} of 1.56 whereas the duration of the plateau of the secondary depolarization (t_{2n}) and the repolarization time (t_{3n}) had higher Q_{10s} of 2.76 and 2.78, respectively. The high Q_{10} of the total duration of the control potential (t_{4n}) is clearly the result of the high temperature dependence of the secondary depolarization. The high temperature dependence of the frequency of control potential is not solely due to the prolongation of the control potential duration (t_4 and t_{4n}) but is also due to an increase in the intercontrol-potential period (t_5 and t_{5n}) which had a Q_{10} of about 2.5.

Cationic Dependence of the Electrical Control Activity:

In the previous section we have shown that the electrical control activity can be abolished by treatments that inhibited the Na pump. In this view the possible contribution of passive sodium and potassium permeabilities which is usually tested by reducing the extracellular Na or K concentration is difficult to demonstrate electrophysiologically. This is so because reducing extracellular K does not only increase the K concentration gradient but also inhibits the Na pump and removing the extracellular Na would lead to depletion of intracellular Na and this in turn would also inhibit the Na pump (for a review see Skou, 1965; Caldwell, 1968). It is therefore difficult to assess whether the cessation of electrical control activity in the absence of Na was the result of Na-pump inhibition or is indicative of the participation of a passive Na-conductance change in the control

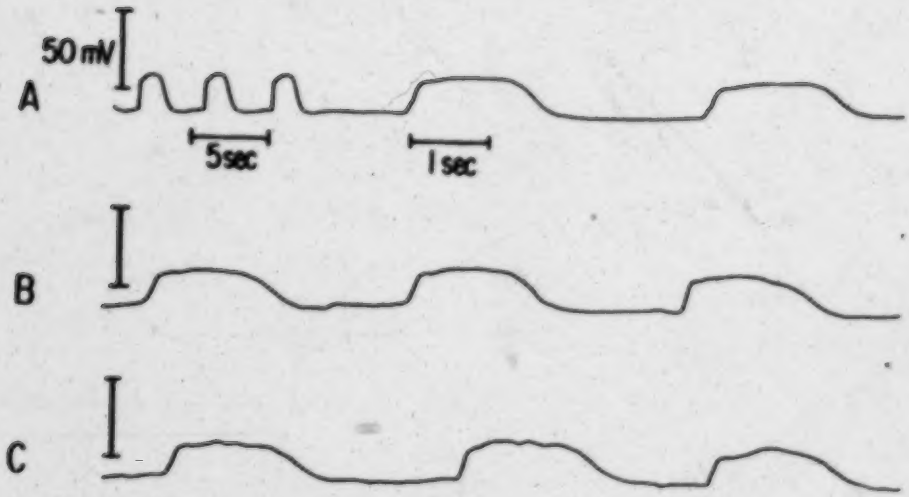

Figure 22. The effect of reducing the external Na to 50% (Li used as substitute) on the electrical control activity A, normal Tyrode solution; B, 30 min after 50% Na (Li)-Tyrode solution; C, normal Tyrode solution. The intestinal control potential was not affected by this treatment.

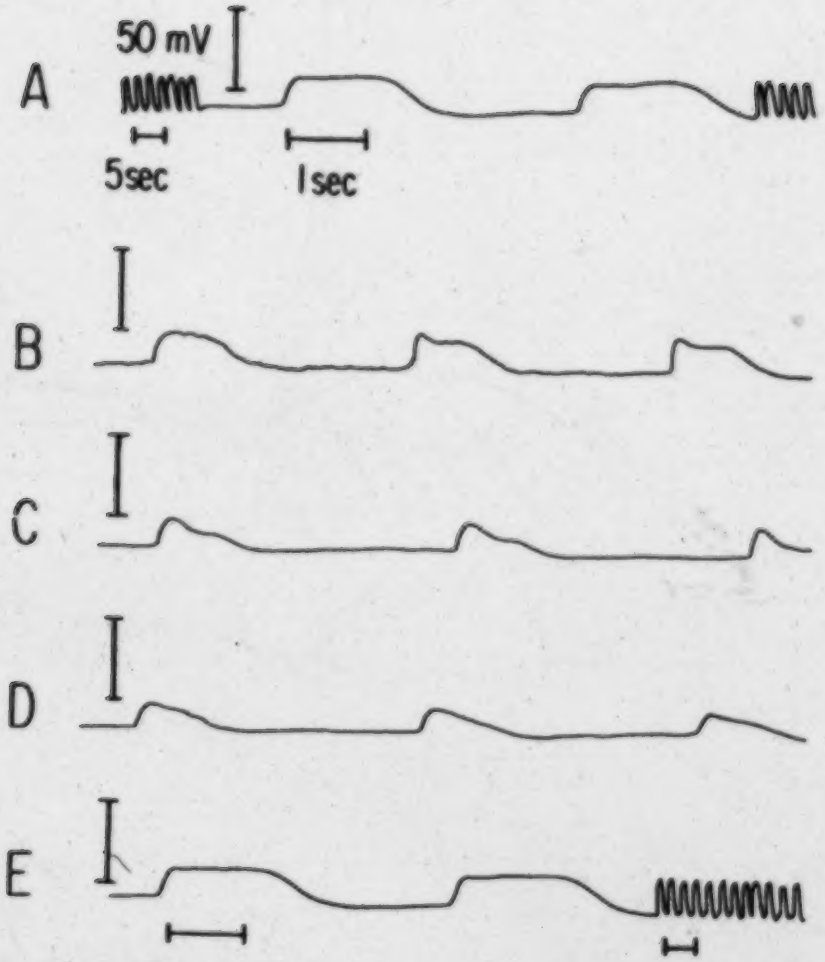

Figure 23: The effect of replacement of 76.5 mM of the NaCl content of normal Tyrode solution by sucrose on electrical control activity. A, normal Tyrode solution; B, C and D, 8, 12 and 18 min after 76.5 mM Na (sucrose) solution; E, 6 min after switching back to normal Tyrode solution.

potential generation, particularly that there are no cations that can replace Na in activating the pump but that cannot utilize the Na-conductance channels.

Nevertheless, as mentioned in the last section, substitution of Li for Na (Na-free solution) and removal of external K reversibly abolished the electrical control activity. To further elucidate the nature of the Na dependence of the control activity we studied the effect of reduction of Na concentration to 13% (19.74 mM Na) and 50% (76.5 mM Na) of its value in normal Tyrode solution using lithium and sucrose as substitutes. Reducing the external Na concentration to 13% using either lithium (4 tissues) or sucrose (3 tissues) completely abolished the electrical control activity in a manner indistinguishable from that in which Na-free (Li) solution abolished it. The resting membrane potential was not significantly changed in Li substituted but decreased to 66.18 ± 0.69 mV (11 penetrations) in sucrose substituted Tyrode solutions. On the other hand, substitution of LiCl for 76.5 mM of the Na Cl of the normal Tyrode solution (solution contained 50% Na but normal Cl) had no effect on the control potential amplitude, configuration or frequency or on the maximum resting membrane potential (Fig. 22). However, the pattern of contractions changed to alternating periods of silence and rhythmic contractions of the same frequency as the control activity.

Replacement of 76 mM of the NaCl of normal Tyrode solution by sucrose (solution contained 50% Na and 54% Cl) produced significant effects on the control potential duration and frequency without affecting its amplitude or rate of depolarization (Fig. 23). The control potential

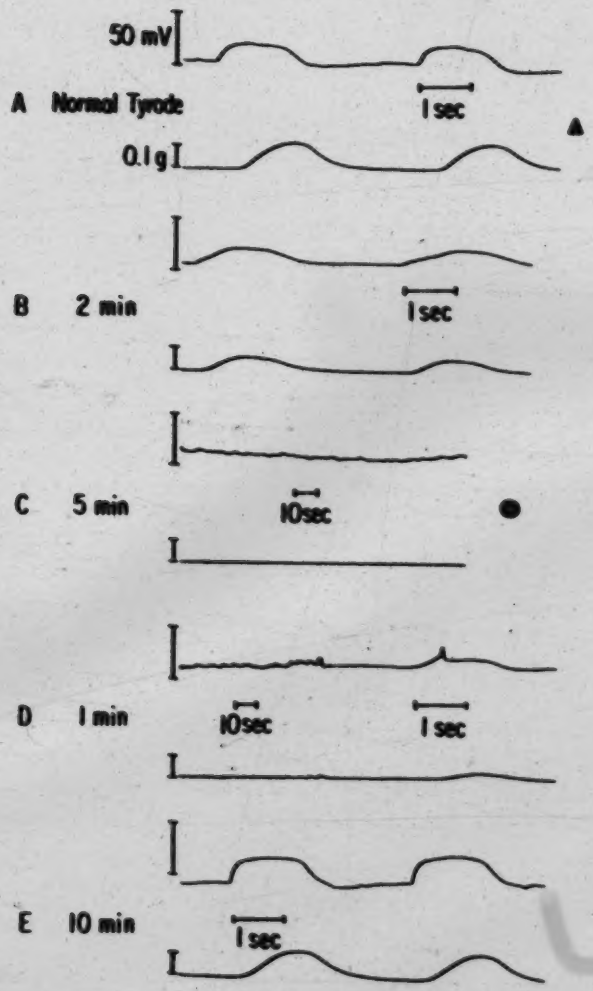

Figure 24: The effect of Ca omission on the electrical control activity. A, normal Tyrode solution, solution changed to Ca-free Tyrode at A; B and C, 2 and 5 min after Ca-free solution, solution switched back to normal Tyrode at ●; D and E, 1 and 10 min after normal Tyrode. A and B, and C and D are parts of continuous tracings from the same penetrations.

duration progressively decreased from its normal value of 1970.1 ± 10.5 to 1222.9 ± 17.1 msec (14 penetrations in 4 tissues) and the frequency decreased from 17.55 ± 0.21 to 14.64 ± 0.27 per min within 10 min of the introduction of the 50% Na (sucrose) solution. Although the control potential amplitude and rate of depolarization were not significantly affected by this treatment, we consistently observed that the plateau phase or the secondary depolarization of the control potential was appreciably depressed in this solution (Fig. 23B-D). There was a hyperpolarization of about 11 mV (54.82 ± 0.31 mV in normal Tyrode versus 66.29 ± 0.86 mV in 50% Na (sucrose)-Tyrode) and the rhythmic contractions disappeared or were greatly reduced in force. These effects were reversed upon the admission of normal Tyrode solution.

The effects of K omission were described in the previous section since they were probably attributable to inhibition of the Na pump.

The omission of external calcium from the bathing solution (9 tissues) increased the maximum resting membrane potential to a value of -38.69 ± 0.57 mV (29 penetrations in 9 tissues). The membrane depolarization was accompanied by a progressive but rapid decrease in the control potential amplitude and both the rates of depolarization and repolarization (Fig. 24B). The electrical control activity completely disappeared within 10 min after calcium withdrawal (Fig. 24C and D). The response activity and rhythmic contractions also disappeared at about the same time. The readmission of calcium caused a gradual recovery of the electrical control and response activities and repolarized the membrane. Although experiments in which the smooth muscle cell

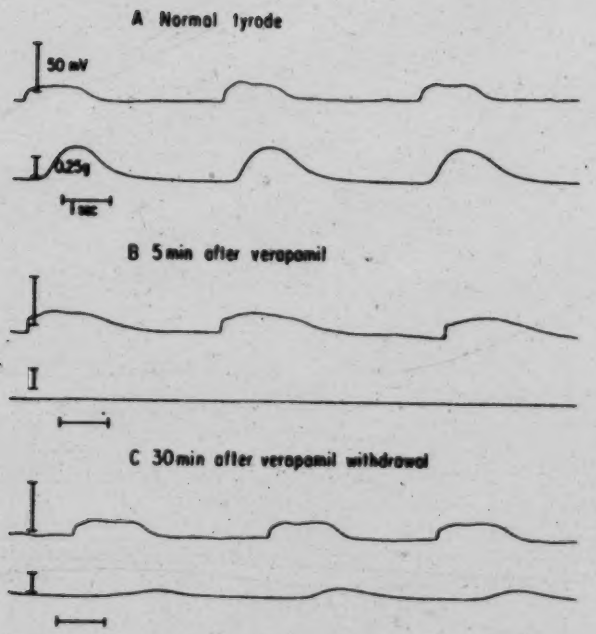

Figure 25: The effect of Verapamil on the electrical control activity. A, normal Tyrode solution; B, 5 min after Verapamil (5 x 10^{-5}M); C, 30 min after normal Tyrode solution. Top and bottom records in each panel represent electrical and mechanical activities, respectively. Notice the incomplete recovery of mechanical activity in C.

membrane is electrically repolarized after it had been depolarized in Ca-free solution have not been conducted, it seems unlikely that the cessation of the electrical control activity was the consequence of depolarization. In most of our experiments it appeared that the recovery of control potentials preceded that of the membrane potential (Fig. 24D).

To investigate whether the sensitivity to calcium omission of the electrical control activity of intestinal smooth muscle cells reflects a current carrying function of calcium ions in the control potential generation, we studied the effects of Verapamil which is a "calcium antagonist" believed to block transmembrane calcium movements (Fleckenstein, Grün, Tritthart and Byon, 1971; Golenhofen and Lammel, 1972; Mayer, van Breemen and Casteels, 1972). Verapamil in a low concentration of 5×10^{-6}M (2 tissues) reversibly abolished the response (spike) activity as well as the rhythmic contraction indicating that these concentrations effectively blocked the transmembrane Ca fluxes. Nevertheless, the electrical control activity persisted even in the presence of a tenfold higher concentration of Verapamil; i.e., 5×10^{-5}M (4 tissues) despite some reduction in the frequency and prolongation of the duration of control potentials (Fig. 25B). The frequency decreased from the normal value of 17.55 ± 0.21 to 14.13 ± 0.26 per min and the control potential duration increased from 1970.1 ± 10.5 to 2483.3 ± 38.4 msec (24 penetrations in 6 tissues). The depolarization time and amplitude of the initial depolarization were not significantly altered by Verapamil (163.3 ± 12.5 msec and 17.0 ± 0.3 mV in Verapamil versus 155.6 ± 4.1 msec and 17.3 ± 0.3 mV in normal Tyrode solution, respectively). There was a slight increase in the amplitude of the

secondary depolarization from 16.9 ± 0.3 to 18.3 ± 0.4 mV. The increase in the control potential duration was due to prolongation of the duration of the secondary depolarization. The maximum resting membrane potential was not significantly affected by Verapamil (-54.50 ± 0.54 versus -54.82 ± 0.31 mV in normal Tyrode). Similar slowing in the frequency, and prolongation of the duration, of the control potential was observed, though not explicitly stated, in guinea-pig stomach by Golenhofen and Lammel (1972). The recovery of contractile activity (and probably response activity) was not complete even 40 minutes after Verapamil withdrawal particularly if high doses were used (Fig. 25C).

Anionic Dependence of the Electrical Control Activity:

The dependence of the intestinal control potential on the external anionic content was investigated by studying the effects of solutions in which NaCl and KCl were replaced by salts of Na and K, the ionic components of which are either less (propionate, isethionate or benzenesulphonate) or more (nitrate) permeant than chloride ions. These chloride-poor solutions contained 3.84 mM Cl since $CaCl_2$ was not replaced by other salts of calcium.

Although not enough penetrations were obtained in the first few minutes after changing the bathing solution from normal Tyrode to propionate-Tyrode to permit statistical analysis, the initial transient effect of this treatment was a slight depolarization and an increase in the amplitude of the control potential. It appeared that the enhancement of the control potential amplitude was mainly due to an increase of the amplitude of the secondary depolarization while

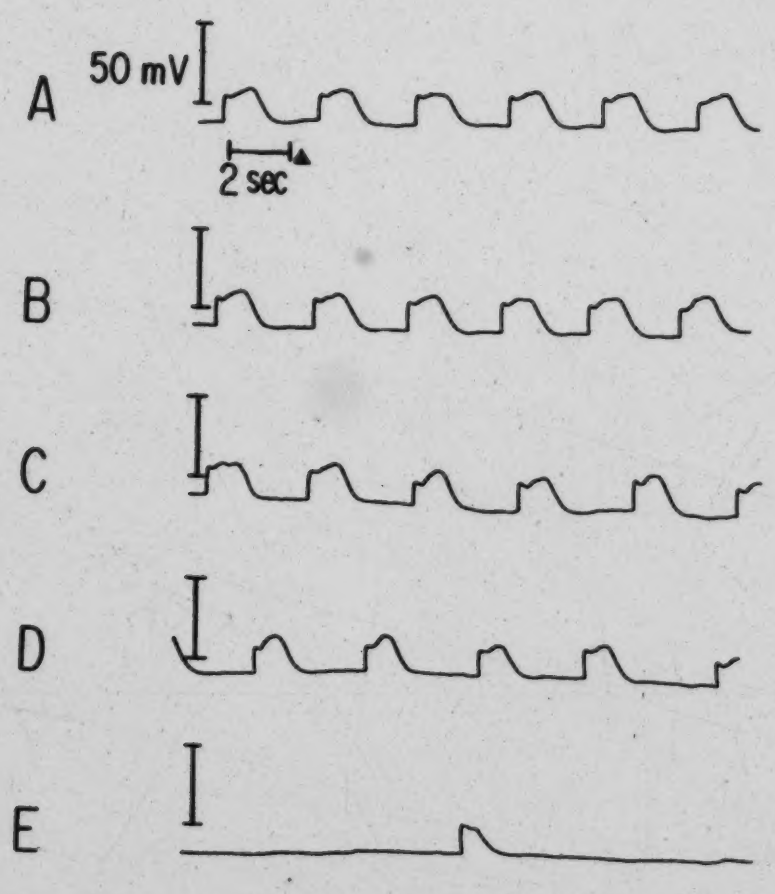

Figure 26: The initial effect of replacement of Cl ion by propionate on the electrical control activity. A, B, C and D represent a continuous record from the same cell while the solution was switched from normal to propionate Tyrode solution; E, record from the same cell 6 min after propionate Tyrode. Notice initial enhancement and subsequent suppression of secondary depolarization.

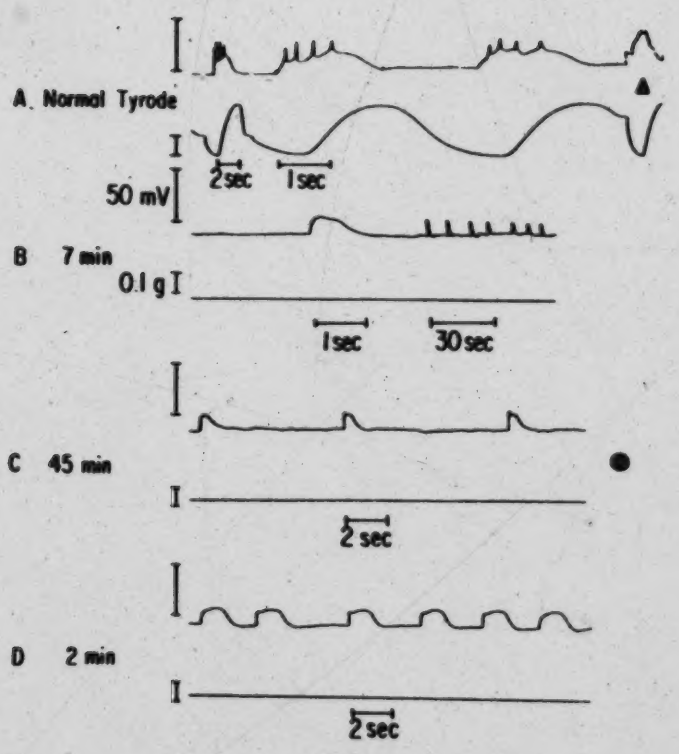

Figure 27: The effect of propionate-Tyrode solution on the electrical and mechanical activity. A, normal Tyrode solution changed at A to propionate-Tyrode; B and C, 7 and 45 min after propionate-Tyrode; D, 2 min after normal Tyrode solution. C and D are parts of a continuous record from the same cell. Top and bottom tracings in each panel show electrical and mechanical activities, respectively.

TABLE 9

The effect of replacement of chloride ions by less permeant anions on the maximum resting membrane potential (RMP) and control potentials

Major anion in solution	RMP (mv)	Control Potential			
		Amplitude (mv)	t_1* (msec)	Duration (t_4) (msec)	Frequency (min^{-1})
Chloride	-54.8 ± 0.3 (218)	17.9 ± 0.2 (208)	155.6 ± 4.0 (171)	1970.1 ± 10.5 (198)	17.6 ± 0.2 (60)
Propionate	-61.2 ± 0.3 (27)	16.0 ± 0.3 (27)	203.0 ± 4.7 (27)	885.9 ± 5.5 (27)	6.3 ± 0.1 (27)
Isethionate	-61.2 ± 0.8 (12)	16.1 ± 0.3 (12)	190.0 ± 12.2 (12)	912.7 ± 11.8 (12)	7.5 ± 0.1 (12)
Benzenesulphonate	-62.0 ± 0.7 (9)	15.4 ± 0.4 (9)	191.1 ± 13.0 (9)	915.6 ± 10.4 (9)	7.5 ± 0.1 (9)

Values expressed as means ± standard errors of the means.

Numbers in parentheses indicate numbers of penetrations

*t_1 is the depolarization time (time from onset to maximum initial depolarization).

that of the initial depolarization did not appear to be appreciably affected. This initial transient effect was observed in all of 3 penetrations in which the electrode remained in the cell during changing medium from normal to propionate Tyrode solution (Fig. 26). This effect was followed by a decrease of the maximum resting membrane potential to a value of 61.19 ± 0.34 mV (27 penetrations in 7 tissues) 7-9 minutes after Cl withdrawal. This hyperpolarization was accompanied by cessation of spike (response) activity and contractions and dramatic changes in the electrical control activity (Figs. 26E and 27B and C); (a) the control potential frequency decreased from the normal value of 17.55 ± 0.21 in normal Tyrode solution to 6.26 ± 0.1 per min, (b) the control potential duration was greatly shortened from 1970.1 ± 10.5 to 885.9 ± 5.5 msec, (c) the shortening of the control potential duration was clearly due to a drastic reduction of the amplitude and duration of the secondary depolarization or even its elimination, and (d) there was only a slight decrease in the amplitude of the initial depolarization from 17.90 ± 0.19 mV in normal Tyrode to 16.04 ± 0.31 mV in propionate-Tyrode solution and a slight but significant increase in the depolarization time from 155.6 ± 4.0 to 203.0 ± 4.7 msec. All these effects persisted as long as the bathing medium was propionate Tyrode (Fig. 27C) and were rapidly reversible upon changing the bathing solution back to normal Tyrode solution (Fig. 27D).

The effects of replacing chloride by the other less permeant anions isethionate (3 tissues) or benzenesulphonate (3 tissues) on the electrical control activity were qualitatively similar in all respects to those of propionate Tyrode. Table 9 summarizes the effects of

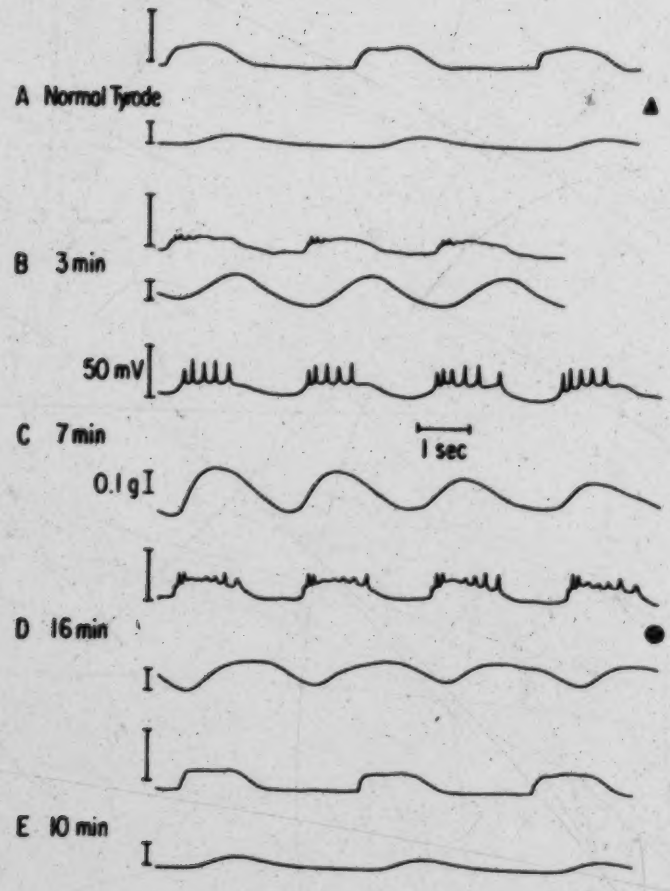

Figure 28: The effect of replacement of Cl ions by nitrate on the electrical and mechanical activity. A, normal Tyrode solution; B, C and D, 3,7 and 16 min after switching from normal to nitrate-Tyrode solution at ▲; E, 10 min after switching back to normal Tyrode solution at ●. Top and bottom tracings in each panel represent electrical and mechanical activityes, respectively.

replacing Cl by less permeant anions on the maximum resting membrane potential and the electrical control activity.

Substitution of the more permeant anion nitrate for chloride (6 tissues) produced effects which were in many respect opposite to those of replacing chloride by less permeant anions. The smooth muscle cell membrane slightly depolarized to -51.04 ± 0.43 mV (23 penetrations) and response activity consisting of bursts of several spikes superimposed on the depolarization phase and plateau of the control potentials appeared in cells which showed no response activity in normal Tyrode solution (Fig. 28B,C and D). In nitrate-Tyrode solution apparently all cells exhibited response activity since in this solution no penetrations in cells which showed only control activity were obtained. The increased response activity was accompanied by an increase in basal tension and occasionally an increase in the force of rhythmic contractions. Although it was difficult to assess the amplitude and configuration of the control potentials under conditions of intense response activity, no observable changes in the parameters of the control potential could be detected. The only observable effect on the electrical control activity was an increase in the control potential frequency from the normal value of 17.55 ± 0.21 to 21.83 ± 0.83 per min. A parallel increase in the frequency of rhythmic contraction occurred. All these effects were readily reversible upon readmission of normal Tyrode solution (Fig. 28E).

DISCUSSION:

A. The Relationship Between the Electrical Control Activity and the Sodium Pump:

Based on his *in vivo* observations that the electrical control activity of canine small intestine was sensitive to temperature, metabolic poisons and inhibitors of the Na pump, Daniel (1962, 1965) suggested that the control potential may be due to oscillations in the activity of an electrogenic Na pump; the "turning off" and "on" of which causes its depolarization and repolarization phases, respectively. Subsequently, Daniel's observations were confirmed *in vitro* in cat small intestinal preparations by Liu, Prosser and Job (1969) who concluded that their observations were consistent with Daniel's hypothesis. However, they reported that inhibition of the Na pump by either ouabain or Na-free solutions did not depolarize the membrane to the potential level at the peak depolarization of the control potential as one would expect if the control potential depolarization was due to turning off of the pump. Furthermore, they showed that intracellular iontophoretic injection of the Na ions increased the control potential amplitude some 3.5 times and suggested that this was due to stimulation of the electrogenic Na pump, presumably by the increase in intracellular Na. Their figure illustrating this effect however, shows that the control potential amplitude increased and decreased *instantaneously* at the beginning and end, respectively, of Na injection and remained constant during the entire period of injection. As pointed out by Bortoff (1972), the intracellular injection of Na would be expected, in terms

of the oscillating electrogenic pump hypothesis, to cause gradual rather than instantaneous changes in the control potential amplitude.

It is obviously crucial to the oscillating electrogenic Na pump hypothesis to demonstrate that the Na pump in the longitudinal smooth muscle cells of the small intestine is electrogenic and capable, under normal conditions, of providing a contribution to the membrane potential at least equal to the amplitude of the control potential (necessary but insufficient conditions). In our studies (Part IV, Chapter 2) we have presented evidence that the Na pump in these smooth muscle cells can operate electrogenically. However, theoretical analysis revealed that, if any reasonable assumptions are made regarding the passive membrane permeabilities, ionic concentrations, pump rate and coupling ratio, the contribution of the activity of the pump cannot exceed a few millivolts and is much smaller than the amplitude of the control potential.

In the studies reported in this chapter, inhibition of the Na pump by withdrawal of K, total replacement of Na by Li, ouabain, dinitrophenol or by cooling (15-18° C) reversibly abolished the electrical control activity but never depolarized the membrane at the time the control potentials first disappeared to the potential level at the peak depolarization of the control potential as predicted from the oscillating electrogenic Na pump hypothesis. In the studies reported in an earlier chapter (Part IV, Chapter 1), the average maximum resting membrane potential was about -55 mV and the mean control potential amplitude was 18 mV. The values of the membrane potential at the time the control activity first disappeared was 8 to 15 mV more negative

than the potential level at peak depolarization of the control potential which was about -37 mV.

The admission of K to Na-rich tissues caused a rapid hyperpolarization and the appearance of control potentials of near normal frequency but much smaller amplitude. The hyperpolarization lasted 10 to 20 min after which time the membrane potential and the control potential amplitude progressively increased to reach values close to those observed in fresh tissues 45 to 60 min after K admission. This finding presents another strong challenge to the oscillating electrogenic Na pump hypothesis of the control potential generation. The membrane potential in Na-rich tissues in K-free solution was about -18 mV (presumably represents the Goldman diffusion potential) and decreased to -75 mV upon K admission. Since this hyperpolarization was sensitive to Na pump inhibition (Part IV, Chapter 2) it reflected the stimulated activity of the electrogenic Na pump. Under these conditions, the 'maximal' stimulation of the pump greatly augmented its contribution to the membrane potential and two alternative possibilities regarding its presumed ability to oscillate existed:

1. The first possibility is that the pump, because of its maximal stimulation (presumably by the high intracellular Na) could not be "turned off" and is "on" all the time; hence, no control potentials should have appeared until it had extruded enough Na and its activity slowed down.
2. The other possibility is that the pump could somehow oscillate despite its maximal stimulation and its turning off and on should have led to the appearance of control

potentials concomitant with membrane hyperpolarization upon K admission. The amplitude of these potentials would be expected to be much larger than normal during maximal hyperpolarization and to decline progressively as the pump activity slows down and the membrane potential increases.

Neither of these two alternate predictions was observed in any of our experiments; instead, control potentials of small amplitude appeared at the time of maximal hyperpolarization and their amplitude gradually increased as the pump activity slowed down and the membrane hyperpolarization diminished. This reciprocal relationship between the rate of pump activity and the control potential amplitude was also observed in fresh tissues upon temporary (about 30 min) withdrawal followed by re-admission of K.

The results of our experiments on the effects of inhibition and stimulation of the Na pump; namely, the discrepancy between the value of the membrane potential at the time the control activity first ceased following pump inhibition and that at the peak depolarization of the control potential, and the appearance of small control potentials upon activating the Na pump in Na rich tissues and the gradual increase in their amplitude as the rate of pump activity slowed down, strongly militate against the oscillating electrogenic Na pump hypothesis.

The oscillating electrogenic Na pump hypothesis is also incapable of providing adequate explanations for some other properties of the intestinal control activity. Firstly, there is strong evidence that the spread of control activity between longitudinal smooth muscle

cells is nondecremental and that bidirectional coupling between the control potentials of adjacent cells (or oscillators) probably via current flow occurs (Sarna, Daniel and Kingma, 1971; Henderson, Duchon and Daniel, 1971; Daniel, Duchon and Henderson, 1972). Secondly, Specht and Bortoff (1972) demonstrated, contrary to earlier belief (Kobayashi, Prosser and Nagai, 1967), that the intestinal control potentials can be electrically excited and entrained by extracellularly applied depolarizing or anodal break currents. Thirdly, Weems, Conner and Prosser (1973) claimed that hyperpolarization increased, and depolarization decreased, the control potential amplitude. To reconcile these properties with the oscillating pump hypothesis one has to assume that the pump activity is voltage dependent and that current pulses could trigger its turning off. Although the voltage dependence of the electrogenic Na pump has not been studied in smooth muscle cells, strong evidence exists that the pump activity in molluscan neurons is independent of the membrane potential (Marmor, 1971).

B. The Temperature Sensitivity of Electrical Control Activity:

The intestinal control potential in most cells appears as a depolarization phase, a plateau and a repolarization phase. In some cells, however, a notch appeared persistently early in the plateau phase particularly at low temperatures. This finding led us to suggest that the control potential may consist of two components, an initial and a secondary depolarization, which probably have different temperature sensitivities (Part IV, Chapter 1). The separation of the extracullularly recorded control potential into two components at low

temperatures has previously been observed by Job (1969). In this chapter the effect of temperature on the intestinal control activity was further investigated mainly in the range between 27° C and 37° C. The amplitudes of both the initial and secondary depolarizations of the control potential were temperature insensitive and the rate of the initial depolarization had a low Q_{10} of 1.56. On the other hand, the duration of the secondary depolarization (or the plateau duration in un-notched control potentials) and the rate of repolarization had appreciably higher temperature coefficients. The temperature sensitivity of the total control potential duration appeared to be mainly the result of the high Q_{10} of the secondary depolarization. The sensitivity of the control potential frequency to temperature is not solely the result of the high Q_{10} of the control potential duration since the time period between control potentials (intercontrol-potential period) also had a high Q_{10}. Our finding that at 27° C most cells exhibited notched control potentials may indicate that the rate of "turning on" of the process responsible for the secondary depolarization is more temperature sensitive than that for the process underlying the initial depolarization; thus cooling would cause sufficient temporal separation to allow notching of the control potential. The maximum resting membrane potential increased by about 2 to 3 mV upon cooling from 37 to 27° C.

Our finding that despite the slight depolarization, cooling from 37 to 27° C did not significantly alter the control potential amplitude is not consistent with the oscillating electrogenic Na pump hypothesis. According to this hypothesis the pump activity contributes some

18 mV (the height of the control potential) and is periodically turned off to produce the control potentials and thus a 10° C cooling would be expected to produce a larger depolarization accompanied by a decrease in the control potential amplitude. The separation of the control potential by a notch into two components particularly at low temperatures is also inexplicable by the oscillating electrogenic pump hypothesis or any other hypothesis that attributes the control potential to a single "on-off" event.

C. The Ionic Dependence of the Electrical Control Activity:

Earlier work (Daniel, 1962, 1965; Tamai and Prosser, 1966; Lui, Prosser and Job, 1969) revealed that the electrical control activity could be abolished in Na-poor and Na-free media. Some of these authors (Daniel, 1962, 1965; Lui, Prosser and Job, 1969) interpreted this effect in the light of the oscillating electrogenic Na-pump hypothesis as being a consequence of pump inhibition resulting from depletion of intracellular Na. This interpretation is difficult to reconcile with the finding of some of these authors (Tamai and Prosser, 1966; Lui, Prosser and Job, 1969) that the membrane potential did not depolarize to the peak of the control potential when the control activity ceased following Na withdrawal. Furthermore, taken in isolation, this interpretation is not the only possible one for the control activity would have also ceased if the control potential depolarization was the result of an inward Na current. That this last possibility is more likely is supported by the study by Job (1969) of the ionic fluxes during the control potential in segments of cat jejunum from which the mucosa and submucosa had been removed. The segments were placed in a closed continuous-flow

chamber which allowed recording of electrical activity by extracellular pressure electrodes. The perfusate was led to a revolving collecting carousel which allowed the collection of 15 radioactive fractions per control potential. This technique suffers from several weaknesses that limit its use as a quantitative tool. For example, the phase lag between control potentials in different cells or regions of the preparation could cause the spread of the change in ionic fluxes associated with control potentials over a wider time interval than that of the control potential. The use of the intact muscle coat might also complicate the flux patterns, for it is known that the control activity originates in the longitudinal muscle cells and spreads electrotonically to the underlying circular layer (Daniel, Honour and Bogoch, 1960; Bortoff, 1961a, 1965; Kobayashi, Nagai and Prosser, 1966; Bortoff and Sachs, 1970). Furthermore, the rhythmic contractile activity of such preparations may add further complications. Other problems can arise from diffusion delays within the tissue and from the dead space between the surface of the tissue and the collection tubes. Nevertheless, Job's results indicated that the depolarization phase of the control potential was associated with an increased Na influx and repolarization with Na efflux. Thus, Job suggested that the control potential depolarization may be due to an increase in Na permeability while repolarization may result from the activity of an electrogenic Na pump (he also showed that the repolarization rate exhibited both a high Q_{10} and an optimum at 37° C).

However, there are obvious inconsistencies between the results of Job's (1969) study and the hypothesis he presented. Firstly, his

electrical recordings show that the control potential consists of two components which could be separated when the temperature was lowered to 31.5° C and his hypothesis does not suggest a mechanism for the second component if the first component was due to an increase in Na permeability. Secondly, although he did not speculate on the time at which the increase in Na permeability is turned off, it is probable that it should last for the major part of the duration of the control potential 'plateau' phase. This is not supported from the analysis of his figure (Fig. 6) illustrating the Na influx during control potential depolarization. It is obvious from this figure that the transient increase in Na influx ended long before the repolarization started, leaving unexplained the depolarized state during the control potential plateau. In this figure it appears that the control potentials consisted of two components and that the transient peak of Na influx corresponded with the first component and is probably not responsible for the sustained depolarization of the second component. Furthermore, in his figures illustrating the Na efflux during the control potential (Figs. 3 and 4) the control potentials again consisted of two components and the short-lived peak of Na efflux corresponded to the termination of the first component rather than to the repolarization phase. It is unlikely that the findings that the Na-influx increase was shorter in duration than the plateau phase and that the Na-efflux increase occurred earlier than the repolarization phase might have been due to any of the weaknesses of the technique for all these weaknesses would tend to spread out the ionic events relative to the phases of the control potential attributable to them. These data could best be explained if

the control potential consisted of two components; the first one is due to a transient increase in Na permeability (increased Na influx) while the second is not likely related to Na movements. The increased Na efflux following the peak influx (and first component) may be due to stimulation of the activity of the Na pump by the increase in internal Na and may or may not contribute to the termination of the first component of the control potential.

In the same paper Job (1969) speculated that the mechanisms by which the control potential is triggered may lie either in a cyclical production of ATP by the mitochondria or changes in ATP/ADP ratio. Pursuing this speculation, he studied the effects of some antibiotics which interfere with mitochondrial metabolism and some inhibitors of ATP synthesis on control potential amplitude and frequency (Job, 1971). Based on these effects (the interpretation of which is subject to some uncertainties) he suggested that the control activity may not be driven by mitochondrial oscillations but it rather requires continued synthesis of ATP. He further postulated that the control potential may result from oscillations in Na permeability. A buildup in ATP concentration at the membrane triggers an increase in Na permeability which leads to the control potential depolarization and to an increase in internal Na concentration. The latter, coincident with a high level of ATP at the membrane, stimulates the Na pump. The pump consumes ATP, the ATP concentration at the membrane falls below the threshold value, the increase in Na permeability is turned off and the membrane repolarizes.

In the present study, the sensitivity of the intestinal control activity to the external Na concentration was confirmed. The

control potentials were reversibly obliterated in Na-free Tyrode solution in which all Na had been replaced by Li and in Na-poor solutions in which all NaCl was replaced by either LiCl or sucrose (solution contained 20 mM NaCl). The cessation of control activity was not accompanied by a depolarization to the level at the control potential peak as would be expected if the control potential depolarization was the result exclusively of turning off of the electrogenic Na pump. The membrane potential at the time the control activity first disappeared was only 2-3 mV lower than the potential at maximum polarization between control potentials in normal Tyrode solution. However, the membrane potential and the electrical control activity were not appreciably altered by replacing 50% of the external Na by Li (chloride concentration was normal) whereas a solution low in both Na and Cl (76.5 mM NaCl was replaced by sucrose) hyperpolarized the membrane and appreciably shortened the control potential duration and suppressed the secondary depolarization or the plateau phase. This difference may reflect the sensitivity of the secondary depolarization of the control potential to the external chloride concentration (see below).

The effects of calcium withdrawal has been a subject of controversy. Daniel (1965) found that in vivo intra-arterial perfusion of a segment of dog small intestine with a Ca-free solution reversibly obliterated the electrical control activity in a few preparations; a Ca-free solution containing 5 mM Na_2 EDTA (disodium ehtylenediamine tetraacetate) markedly depressed or abolished this activity. The effect of the EDTA-containing solution could not be reversed by increasing the Mg concentration. In isolated strips of longitudinal

muscle of cat small intestine decreasing the Ca concentration in the bathing fluid to 10% of its normal value seriously depressed the control potential amplitude and frequency and decreased the resting membrane potential (Tamai and Prosser, 1966). However, in another *in vitro* study on strips of the entire muscle coat of cat small intestine, Liu, Prosser and Job (1969) reported that, in Ca-free solutions, the control potentials "continued with unaltered amplitude although the rate of rise and fall of potential were less than normal." These authors argued that the reduction in the control potential amplitude was due to a decline in the prepotentials. In our studies the omission of Ca from the medium consistently decreased the rate of rise and fall and then abolished the control potentials within a few minutes. The resting membrane potential decreased by about 18 mV. These effects were reversed upon the readmission of Ca; the control activity reappearing before the membrane potential had time to repolarize. The reappearance of control activity before appreciable membrane repolarization occurred renders unlikely the possibility that the cessation of the control activity upon Ca withdrawal was a consequence of depolarization rather than a reflection of the dependence of the control potential on calcium ions.

To test whether calcium ions play any role as carriers of transmembrane current during the control potential we studied the effects of Verapamil (iproveratril, isoptin) on the electrical activity of small intestinal smooth muscle. Verapmil is a "calcium antagonist" believed to block transmembrane calcium movements (Fleckenstein, Grün, Tritthart and Byon, 1971; Golenhofen and Lammel, 1972; Mayer, van Breemen

and Casteels, 1972). In our preparations this agent completely abolished the response activity (spiking) and contractions without appreciably altering the control activity even at higher concentrations than those needed to suppress the spikes and contractions. The persistence of control potential of normal amplitudes, despite a slight reduction in their frequency and prolongation of their duration, denies calcium ions any role as current carriers in the generation of the control potential and led us to conclude that one or more of the ionic currents responsible for the control potential generation, although not usually carried by Ca ions, is dependent on the presence of external calcium. Our observation that the control activity ceased if the external Na concentration was reduced below 20 mM without appreciably changing the membrane potential strongly implicates Na ions as the current carriers during the initial deporalization of the control potential. This is supported by Job's finding (1969) that this phase of the control potential was associated with an increase in Na influx. Thus the initial event in the control potential generation may be a Ca-dependent transient increase in sodium permeability, the turning on of which is responsible for the initial depolarization and its turning off may, at least partly, result in the notch.

The dependence of intestinal electrical control activity on external anions was previously studied in two papers only. In the in vivo experiments of Daniel in 1965, the intra-arterial perfusion of chloride-poor Krebs' solution (9.6 mM chloride) in which most of the chloride was replaced by nitrate, sulphate or methylsulphate produced no effects on the control potentials "beyond those produced by Krebs-

Ringer solution itself." In an *in vitro* study, Liu, Prosser and Job (1969) reported that substitution of Na propionate for NaCl did not affect the control potential amplitude or frequency or the resting membrane potential even 90 min after chloride withdrawal. Thus they concluded that chloride ions do not play a significant role in the control potential generation. However, in our studies the electrical control activity of rabbit jejunal smooth muscle cells exhibited a strong anionic dependence. Decreasing the external chloride concentration from 142 to 4 mM by replacement of Na and K chlorides by Na and K propionates, isethionates or benzenesulphonates initially depolarized the membrane slightly and increased the amplitude of the secondary, but not the initial depolarization. This transient effect was followed by an increase in the maximum resting membrane potential (hyperpolarization), a drastic shortening of the duration of the control potentials and decrease in their frequency. The shortening of the control potential duration was clearly due to the elimination or drastic reduction of the secondary depolarization phase of notched control potentials or the plateau phase of un-notched ones. The amplitude and rate of rise of the initial depolarization were only little affected. The common feature shared by these three anions is that they are believed to be less permeant than chloride ions (Hutter and Noble, 1960; Goodford and Lüllmann, 1962; Horowicz, 1964; Grundfest, 1967; Rang and Ritchie, 1968a; Casteels, 1971). On the other hand, replacement of Cl by nitrate, an anion believed to be more permeant than Cl (Rang and Ritchie, 1968a,b) slightly decreased the maximum resting membrane potential and increased the control potential frequency. Although the parameters of the control

potential could not be assessed accurately, due to the intense spiking superimposed on the control potentials in nitrate-Tyrode solution, they did not seem to be appreciably altered by this treatment. Obviously the effects of replacement of chloride by less permeant anions were not observed in the *in vivo* studies of Daniel (1965) on dog small intestine or the *in vitro* experiments of Liu, Prosser and Job (1969) on segments of the entire muscle coat of cat intestine. The effects we observed in rabbit jejunal longtudinal muscle strips were so consistent and obvious to preclude the possibility that they were overlooked by these workers had they occurred. The solutions used by these authors contained more chloride than those used in our studies but the difference is too small to explain the discrepancy of results. Possibly this discrepancy may reflect species difference or more likely their use of thicker preparations where depletion of chloride may not have been complete. Nevertheless, our findings clearly demonstrate the selective sensitivity of the secondary depolarization in notched, and the plateau phase in un-notched, control potentials to substitution of less permeable anions for chloride ions. Thus we suggest that

(1) the plateau phase of the un-notched control potential corresponds to the secondary depolarization of the notched control potential, the only difference between the two control potentials lies in the extent to which the initial and secondary depolarizations are fused together. This is substantiated by the similarities in the temperature dependence between the plateau and repolarization phase of un-notched control potentials and the secondary depolarization

of notched control potentials. Therefore, the plateau and the repolarization phase of un-notched control potentials also will be designated as "secondary depolarization,"

(2) chloride ions carry the transmembrane current during the secondary depolarization; and

(3) a transient increase in chloride permeability which either accompanies or shortly follows the increase in Na permeability is responsible for the secondary depolarization; the turning off of which is responsible for the repolarization phase. An increase in Cl permeability would be expected to increase the passive outward negative chloride current and to depolarize the membrane since the chloride equilibrium potential in smooth muscle cells is about -25 mV (Casteels, 1969, 1970, 1971; Brading, 1971).

In view of this hypothesis one can easily see that the initial increase in the amplitude of the secondary depolarization concomitant with the slight membrane depolarization upon replacement of Cl by less permeant anions may have been due to the increase in the chloride concentration gradient across the membrane and the shift of E_{Cl} towards more positive value. As redistribution of Cl ions occurred, which appears to be fast indeed, the secondary depolarization gradually disappeared concomitant with membrane repolarization. This hypothesis can also explain our finding that replacement of 76 mM of the NaCl by sucrose, but not by Li Cl, significantly shortened the control potential duration and decreased the amplitude of the secondary depolarization.

It is important to emphasize here that the effects of substitution of less permeant anions on the control potential present a strong challenge to all of the hypotheses previously postulated to explain its ionic basis. It is known that the contribution of the electrogenic Na pump to the membrane potential increases upon replacement of Cl by less permeant anions (for example Rang and Ritchie, 1968 a,b; Taylor, Paton and Daniel, 1970) by reducing the current carried by Cl (or smaller) ions which normally short-circuits the potential generated by the pump. If the control potential was the result of turning off and on of the electrogenic Na pump, then one would expect that reducing the Cl short-circuiting current should decrease the maximum resting membrane potential and increase the control potential amplitude. In our experiments, no increase in the control potential amplitude was observed despite a 5-8 mV decrease in the maximum resting membrane potential. Similarly the effects of less permeant anions on the control potential are also inexplicable by either of the hypotheses proposed by Job in 1969 (an increase in P_{Na} followed by increased electrogenic pumping) and in 1971 (turning on and off of a P_{Na} increase).

In summary, our findings present a strong challenge to all previously published hypotheses concerning the ionic mechanisms of the control potential generation and strongly point towards a new hypothesis. The control potential is visualized as consisting of an initial depolarization followed by a secondary depolarization. Both phases are often fused together so that the control potential appears as a depolarization phase, a plateau and a repolarization phase but occasionally (particularly

at lower temperatures) they may be temporally separated so that a notch appears early in the plateau phase. The notch represents the turning off of the initial depolarization before the full activation of the secondary depolarization. The initial depolarization results from a Ca-dependent increase in Na permeability while the secondary depolarization results from a transient increase in Cl permeability which accompanies or shortly follows, but is slower than, the increase in Na permeability (Fig. 29).

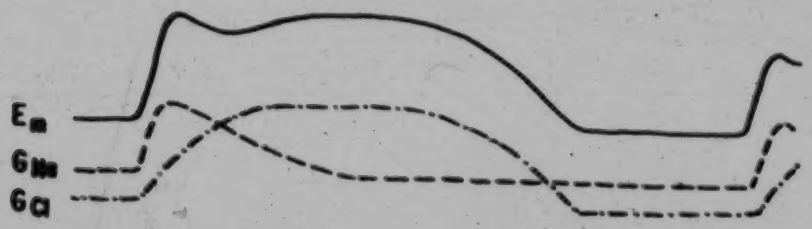

Figure 29: The proposed ionic mechanisms underlying the intestinal control potential.

The next obvious question to be raised is concerned with the mechanism(s) involved in triggering the permeability changes underlying the intestinal control potential. We have shown (Part IV, Chapter 1) that while the period between control potentials (the intercontrol-potential period) remained equipotential in most cells, slow depolarizations of up to 6 mV were observed between each two successive control potentials in some cells. The physiological function of these "inter-control-potential depolarizations," if it exists is unknown, nor is their ionic mechanism. It is, however, interesting to speculate that these depolarizations may serve as the trigger for the initial event in

the control potential generation; i.e., the increase in Na permeability in the same manner the slow diastolic depolarizations of the spontaneously active cardiac muscle fibers trigger the cardiac action potential. Such a triggering mechanism is supported by the recent discovery of Specht and Bortoff (1972) that the control potentials could be electrically excited and entrained by depolarizing and 'anode break' currents. This suggestion would also explain the finding that the control potential frequency could be modulated by displacement of the membrane potential; a decrease on hyperpolarization and an increase on depolarization (Weems, Conner and Prosser, 1973).

In terms of this suggestion every longitudinal smooth muscle cell which is capable of producing these slow 'diastolic' depolarizations can produce control potentials at a frequency determined by its characteristic rate of intercontrol-potential depolarizations and its excitability. Bidirectional coupling between the "oscillator cells" may occur *via* currents flowing from one cell to the other as suggested by Henderson, Duchon and Daniel (1971). The frequency of the control potentials in a preparation *in vitro*, or a region of the intestine *in vivo*, may be determined by the frequency of the control potentials in the cell, or group of cells, possessing the highest rate of intercontrol-potential depolarization and/or highest excitability as depicted from the bidirectionally coupled relaxation oscillator model for the control activity of the small intestine. Our finding that the frequency of control potentials could be decreased by replacing chloride ions by less permeant, and increased by more permeant, anions implicates chloride ions in the generation of intercontrol-potential depolarizations. It is possible

that these depolarizations may result from an increase in Cl permeability distinct from the one that underlies the secondary depolarization of the control potential. One common feature of the intercontrol-potential period and the secondary depolarization, besides their sensitivity to chloride substitution, is their sensitivity to temperature which may reflect the possibility that the membrane permeability to chloride ions may be regulated by cellular metabolism.

The transient increase in chloride permeability that underlies the secondary depolarization of the control potential may, indeed, be triggered by the initial depolarization that results from the increase in Na permeability. This assumption is based on our finding that in Na-poor and Na-free solutions no membrane potential oscillations attributable to the transient increase in Cl permeability could be recorded. The possibility that the increase in intracellular Na following the increase in Na permeability may trigger the increase in Cl permeability is unlikely since the duration of the control potentials recorded from recovering Na-rich tissues was not different from that of normal tissues. It is possible, then, that the turning on and off of the increase in Cl permeability may be triggered by the turning on and off of the initial depolarization. It is interesting to mention at this point that a voltage-dependent increase in P_{Cl} has been shown to underly the initial rapid repolarization that precedes the action potential plateau in cardiac action potential (Dudel, Peper, Rüdel and Trautwein, 1967; Peper and Trautwein, 1968). An increase in P_{Cl} of cardiac muscle fibers already depolarized by the action potential upstroke would hyperpolarize the membrane because the E_{Cl} in these fibers is about -80 mV (Dudel, Peper,

Rüdel and Trautwein, 1967) while its increase in smooth muscle cells at the normal resting potential would depolarize the membrane since E_{Cl} in these cells is about -25 mV (Casteels, 1969; Brading, 1971).

One important test to distinguish whether the control potential is the result of currents generated by the electrogenic Na pump or to passive currents resulting from ionic permeability changes is the measurement of the transmembrane resistance during the electrical control activity. It is unfortunate that the techniques available for such measurements in smooth muscle cells are either difficult or subject to some criticisms. The use of two intracellular microelectrodes, one to inject current and one to record the resultant potential change, is obviously difficult due to the small size and movement of cells. The use of a single microelectrode for both purposes is unreliable (see Holman, 1968). The Abe and Tomita technique (Abe and Tomita, 1968) of applying current with extracellular electrodes and recording the electrotonic potential intracellularly is more reliable particularly in tissues where low resistance contacts (usually assumed to be the nexuses) between smooth muscle cells exist. In tissues where no such contacts have been found, like the longitudinal muscle layer of the small intestine of the dog (Henderson, Duchon and Daniel, 1971; Daniel, Duchon and Henderson, 1972), rabbit (unpublished observation from this laboratory) and guinea pig (Gabella, 1972, 1973), the uncertainties about the current pathways may cast some doubts on the interpretation of the results of such experiments. The application of the double sucrose-gap technique to measurement of the membrane resistance changes associated with an electrical event (like the control potential) suffers not only from the same criticisms as the Abe and Tomita technique but

also from weaknesses resulting from the use of extracellular recording electrodes. Since the extracellularly recorded electrotonic potential is the summed response of the thousands of cells at the node to the extracellularly applied current pulse, it is essential that all of these cells be simultaneously in the same phase of the electrical event during which the resistance changes are to be measured. The simultaneous occurrence of the control potential in the smooth muscle cells of a preparation is not likely since there is an aborally increasing phase lag in the longitudinal spread of intestinal control activity (see, for example, Daniel and Chapman, 1963).

Nevertheless, using the double sucrose gap two groups of investigators attempted to determine the membrane resistance changes associated with the control potential. In longitudinal strips of rabbit ileum (longitudinal + circular muscle) Taylor and Mills (1970) and Mills and Taylor (1971) found that the size of the electrotonic potential was smaller at the plateau and repolarization phase of the control potential than during the intercontrol-potential period. These authors concluded that an increase in membrane conductance may underly the control potential depolarization. On the other hand, in a more recent study (of which only an abstract is so far available) on 'pure' longitudinal muscle strands, Weems, Conner and Prosser (1973) claimed to have found no change in the size of electrotonic potentials during the control potential and concluded that the control potential "need not result from ionic conductance changes ... but may result from ionic currents produced by a cyclic electrogenic sodium pump." Their apparent failure to demonstrate conductance changes associated with the control

potential may be due to their use of 'pure' longitudinal muscle strands in which it is probable that appreciable phase lag in, or even desynchronization of, the control activity in various regions in the node existed. This is likely since it has been shown that the integrity of the circular muscle layer in an isolated preparation is necessary for the synchrony of the electrical control activity in the longitudinal fibers (Kobayashi, Nagai and Prosser, 1966). Although the results of Taylor and Mills (1970) and Mills and Taylor (1971), which may be more reliable, are consistent with our hypothesis, it is abundantly clear that they need be confirmed with the Abe and Tomita technique.

SUMMARY AND CONCLUSIONS:

Based on our observations in this and previous chapters we concluded that neither of the hypotheses reported in the literature to explain the ionic basis of the intestinal control potential is capable of explaining our observations and some of the important findings of other workers. Our results also strongly suggest an alternative new hypothesis which is consistent with most, if not all, of the known properties of the intestinal electrical control activity. In terms of this hypothesis the control potential results from two permeability changes occurring in sequence. The first is a Ca-(and probably voltage) dependent transient increase in Na permeability which causes the initial depolarization phase of the control potential. The second is a slower transient increase in Cl permeability which is responsible for the plateau phase of un-notched control potentials or the secondary depolarization of notched control potentials. The turning on and off of the

Cl-permeability increase may be triggered by the changes in the membrane potential caused by the preceding change in Na permeability.

A. Inconsistencies with the Oscillating Electrogenic Na-Pump Hypothesis of Daniel (1965):

1. Although the Na pump was shown to be electrogenic in rabbit jejunal smooth muscle cells, theoretical analysis led us to conclude that, if reasonable assumptions are made about ionic membrane permeabilities and concentration gradients and the rate of pump activity and its coupling ratio, it is unlikely that the pump is capable under normal conditions of providing a contribution to the membrane potential that is close to the amplitude of the control potential.

2. Inhibition of the Na pump by a variety of procedures never depolarized the membrane at the time the control activity ceased to the peak of the control potential depolarization (our results and also those of Liu, Prosser and Job, 1969).

3. K admission to Na-rich tissues, or to fresh tissues temporarily deprived of K, caused the appearance of control potentials of small amplitude but normal frequency. The control potential amplitude progressively increased as the electrogenic Na-pump activity slowed down.

4. The control potentials were abolished in Ca-free solutions. This effect is not likely to be merely due to the accompanying membrane depolarization.

5. Replacement of Cl by less permeant anions did not enhance the amplitude of the control potentials although evidence exists that

such treatment increases the potential generated by the electrogenic Na pump by reducing the chloride short-circuiting current.

6. The control potentials recorded from some cells showed a notch early in the plateau phase particularly at low temperatures (reported also by Tamai and Prosser, 1966 and Liu, Prosser and Job, 1969).

7. The control potential amplitude has a low temperature coefficient.

8. A study of the ionic fluxes during the control potential showed an increase in Na influx associated with the depolarization phase followed by a very transient increase in Na efflux which ended long before the start of the repolarization phase (Job, 1969). This hypothesis predicts a decrease in Na efflux during the depolarization and plateau phases and an increase in Na efflux during repolarization phase.

9. The intestinal control potential is electrically excitable, and the electrical control activity can be entrained by both depolarizing and anodal break currents (Specht and Bortoff, 1972). Hyperpolarization increases, and depolarization decreases, the control potential amplitude (Weems, Conner and Prosser, 1973). There is no reason to believe that the activity of the electrogenic Na pump is voltage dependent (see Marmor, 1971).

10. Despite claims to the contrary (Weems, Conner and Prosser, 1973), there is indication that the membrane conductance is higher than its "resting" value during the plateau and repolarization phase of the control potential (Taylor and Mills, 1970; Mills and Taylor, 1971).

B. Inconsistencies with the Na-Permeability Increase-Electrogenic Pumping Hypothesis of Job (1969):

1. The results of the original experiments on the ionic fluxes associated with the control potential (Job, 1969) on the basis of which this hypothesis was developed are in serious contradiction with the hypothesis. Although not mentioned explicitly, the hypothesis implies that not only the depolarization phase is caused, but also that the plateau phase is maintained, by the increase in Na permeability while the repolarization is due to increased activity of electrogenic Na extrusion. From the figures in Job's paper, it is clear that the increased Na influx was associated only with the depolarization phase and ended long before the end of the plateau phase. The increase in Na efflux, which was taken as evidence for the repolarization being due to increased electrogenic pumping occurred immediately following the initial depolarization and coincided with the notch rather than with the repolarization phase.

2. Notching in the control potentials of some cells cannot be explained by this hypothesis even if one assumes that the notch is due to turning off of the Na-permeability increase for if it was so the maintained depolarization at the plateau phase would remain unexplained.

3. The elimination, or drastic shortening, of the plateau or secondary depolarization of the control potential upon replacement of chloride by less permeant, but not by more permeant anions is not explicable in terms of this hypothesis. If the control potential repolarization was due to increased electrogenic pumping, then reduction of the shunting effect of the Cl current on the pump-generated potential should

increase the membrane potential and the control potential amplitude. Although the membrane potential increased, there was no enhancement of the control potential amplitude upon replacing Cl by less permeant anions.

4. It has been demonstrated by Taylor and Mills (1970) that the membrane conductance during the repolarization phase was higher than the resting conductance between control potentials.

C. Inconsistencies with the Oscillating Na-Permeability Hypothesis of Job (1971):

1. Notching of the control potentials recorded from some cells is impossible to explain by any hypothesis that attributes the control potential to a single "on-off" process.

2. The effects of replacing Cl by less permeant anions on the control potential configuration and frequency are also impossible to explain.

3. In the study of the ionic fluxes associated with the control potential by the author of this hypothes (Job, 1969), the increase in Na influx ended long before repolarization started. The control potential plateau was not associated with an increased Na influx nor was the repolarization phase with a decreased Na influx.

4. The differences in the temperature sensitivity between the rate and amplitude of the initial depolarization on one side and the plateau duration and rate of repolarization (i.e., the secondary depolarization) on the other side are also not readily interpretable in terms of this hypothesis.

D. The Present Hypothesis:

The hypothesis suggested in this chapter is consistent with all but one of the known properties of the intestinal control potential. It can explain the following properties:

1. Notching as being the reflection of the operation of two sequential processes in the generation of the control potential.

2. The sensitivity to external Na in a manner incompatible with the oscillating electrogenic Na pump hypothesis.

3. The cessation of the electrical control activity in Ca-free solution and their persistence in the presence of agents which block transmembrane Ca movements.

4. The differential temperature sensitivity of the initial and secondary depolarizations.

5. The selective suppression of the secondary depolarization upon replacement of Cl by less, but not by more, permeant anions.

6. The changes in the membrane conductance during the control potential measured by Taylor and Mills (1970) and Mills and Taylor (1971).

7. The changes in ionic fluxes measured by Job (1969) which fit nicely with our hypothesis. The Na influx increased during the depolarization phase and came back to its "resting" level at the notch of the control potential. There was a very transient increase in both Na efflux and K influx at the notch which was not maintained during the secondary depolarization. This may reflect stimulation of the pump immediately following the Na influx during the depolarization phase. It may or may not contribute to the initial small repolarization

component of the notch. The time courses of both the Na influx increase and the Na efflux (and K influx) increase are too short to explain the plateau and repolarization phases of the control potential, respectively.

8. The ability of depolarizing and anodal break currents to excite and entrain the control potentials (Specht and Bortoff, 1972) can also be explained.

9. The hypothesis also predicts that hyperpolarization would increase, and depolarization would decrease, the control potential amplitude as reported by Weems, Conner and Prosser (1973).

10. The bidirectional coupling of the control potentials in the cells of an intestinal smooth muscle preparation *in vitro* or in neighbouring oscillators *in vivo* can also be readily explained.

The only property that remains unexplained by this hypothesis is the cessation of the electrical control activity upon inhibition of the Na pump. It is possible that the activity of the Na pump may be involved in the triggering of the control potential, may be necessary for the turning off of the Na-permeability increase or may play a permissive role that is as yet unclear. Further work is need to elucidate the relationship between the pump activity and the electrical control activity.

CHAPTER 4

THE ELECTROPHYSIOLOGICAL BASIS OF THE MOTOR INHIBITORY EFFECT OF ADRENALINE ON RABBIT SMALL INTESTINAL SMOOTH MUSCLE

INTRODUCTION:

Inhibition of intestinal motor activity by sympathomimetic amines has been shown to be mediated by both alpha and beta receptors (Ahlquist and Levy, 1959; Levy, 1959; Furchgott, 1960; Bailey, 1965; Rossom, 1965; Rossom and Mujić, 1965; Lum, Kermani and Heilman, 1966; Reddy and Moran, 1968). Studies on the electrophysiological basis of this inhibition have been conducted mainly on the guinea-pig taenia coli. In this smooth muscle adrenaline abolished the spontaneous spike discharge and occasionally hyperpolarized the cell membrane (Bülbring, 1957; Burnstock, 1958). The degree of hyperpolarization depended on the membrane potential prevailing at the time of administration; it was greater for cells with low membrane potential and did not occur when this was already -65 to -70 mV (Bülbring and Kuriyama, 1963b). The cessation of spontaneous spike discharge usually preceded, and did not seem to be a consequence of, the hyperpolarization (Burnstock, 1958; Bülbring and Kuriyama, 1963b).

The adrenaline-induced hyperpolarization has been attributed to stimulation of an electrogenic Na pump (Burnstock, 1958) or to reduction of Na permeability through fixation of calcium at the membrane (Bülbring and Kuriyama, 1963b). However, more recent studies (Bülbring and Tomita, 1969b; Ohashi, 1971) suggested that adrenaline may increase

both K and Cl permeabilities. The cessation of spike discharge by adrenaline has been suggested to be the result of inhibition of the carrier mechanism for the ion concerned in the spike generation (Burnstock, 1958) or to suppression of the pacemaker depolarizations (Bülbring and Tomita, 1969c). Studies on the effect of adrenaline on ionic fluxes have yielded contradictory results. In the studies of Born and Bülbring, (1956), Hütter, Bauer and Goodford (1963) and Bülbring, Goodford and Setekleiv (1966) adrenaline increased the rate of ^{42}K uptake and had a small and variable effect on ^{42}K efflux. Nagasawa (1963) and Jenkinson and Morton (1965, 1967) reported an increase while Setekleiv (1970) found a decrease in ^{42}K efflux upon treatment with adrenaline. In a recent study by Banerjee (1972) adrenaline did not cause any significant change in either efflux or influx of ^{42}K but the uptake experiments showed indications of increased influx due to the action of this drug. Furthermore, adrenaline was found not to increase transmembrane Cl movements (Jekinson and Morton, 1967). The question of the receptors involved in the effects of adrenaline has been studied by Bülbring and Tomita (1969c) and Davis (1970) who found that the increase in K permeability (and consequently the hyperpolarization) is an alpha action while suppression of the spontaneous spike discharge is due to stimulation of the beta receptors.

Studies on the electrophysiological effects of adrenergic drugs on other gastrointestinal smooth muscles are few and were conducted with extracellular recording techniques. Intra-arterial infusion of adrenaline into segments of the small intestine of the dog in vivo inhibited response activity (spiking) in low doses while higher doses

decoupled the electrical control activity; still higher doses abolished the control potentials (Daniel, Carlow, Wachter, Sutherland and Bogoch, 1959; Daniel, Wachter, Honour and Bogoch, 1960; Daniel, 1966). On the other hand, the in vitro studies of Bortoff (1961b), van Harn (1963) and Small and Weston (1971) showed that catecholamines suppressed spiking without affecting the control potentials.

The purpose of the work presented in this chapter is to study the effects of adrenaline on the membrane potential and spontaneous electrical activity of isolated longitudinal strips of rabbit jejunum using intracellular microelectrodes. The study was stimulated by (a) the hypothesis that the control potential of small intestinal smooth muscle may result from sequential turning off and on of the electrogenic Na pump (Daniel, 1962; 1965) and that adrenaline may stimulate this pump (Burnstock, 1958) and (b) the fact that the guinea-pig taenia coli which is the only gastrointestinal muscle studied by this technique shows enough peculiarities in its electrophysiological properties to make its acceptance as a prototype of intestinal smooth muscle unjustifiable (Daniel, 1973).

RESULTS:

The electrical activity of the small intestinal smooth muscle cells (Part IV, Chapter 1) consists of three components: (a) the control activity which consists of repetitive dipolarization-repolarization cycles (control potentials or slow waves) of about 18 mV average amplitude and 2 sec duration (time from onset of depolarization to end of repolarization) occurring at an average frequency of about 17.6 per min and which arise

from a maximum resting membrane potential (potential level at maximum polarization between control potentials) of -54.8 mV, (b) the response activity which consists of the bursts of one to several spikes which often appear on the depolarization phases of the control potentials in some, but not all cells, and (c) the prepotentials which are slow depolarizations of up to 4 mV which occur superimposed on the control potential depolarization preceding the spikes. Each complex of the electrogram (the control potential with its superimposed burst of spikes) is associated with a contraction. Furthermore, a few cells persistently exhibited 'diastolic' slow depolarizations of up to 6 mV between control potentials (intercontrol-potential depolarizations). Our studies on the configuration of the intestinal control potential (Part IV, Chapter 1) revealed that while in most cells the control potential was monophasic, consisting of a depolarization phase, a plateau and a repolarization phase, some cells exhibited a small notch early in the plateau phase. We subsequently showed that the control potential consisted of two components; an initial depolarization, caused by a Ca-dependent transient increase in Na permeability, and a secondary depolarization due to a transient increase in Cl permeability which accompanies, or shortly follows, but is slower than, the Na-permeability increase. The two components are fused together so that the control potential appears monophasic, but occasionally they may be fused to a lesser extent so that a notch appears early in the plateau phase.

One minute after treatment with 1×10^{-6} M adrenaline (15 tissues), the bursts of spike discharge (response activity) and the prepotentials that precede the spikes disappeared without any appreciable change in

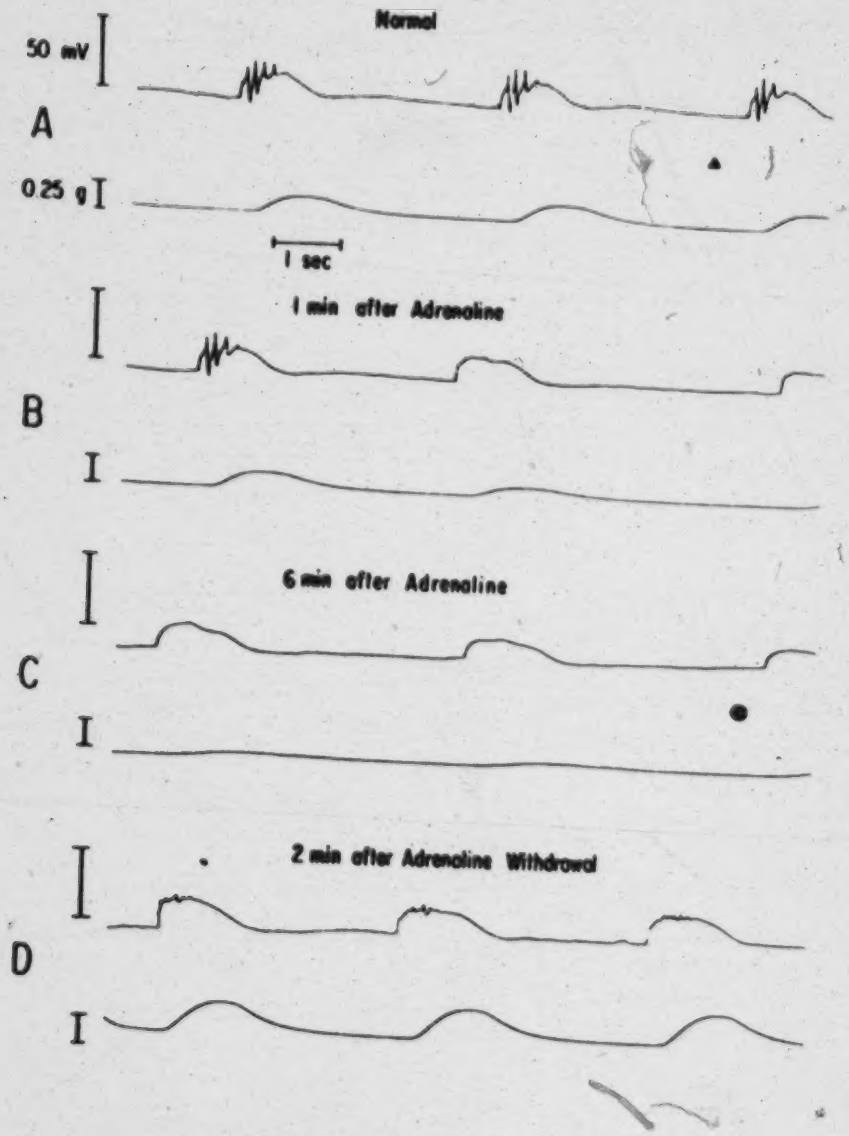

Figure 30: The effect of adrenaline (1×10^{-6}M) on the electrical and mechanical activity. A, normal Tyrode solution was changed to adrenaline containing Tyrode at A; B and C, 1 and 6 min after adrenaline-Tyrode, respectively; D, 2 min after switching back to normal Tyrode. Top and bottom records in each panel represent electrical and mechanical activities, respectively.

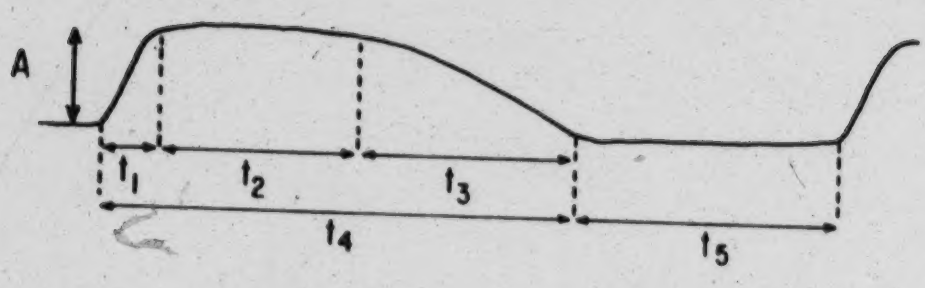

Figure 31: The parameters of the un-notched control potential used in the analysis of the effect of adrenaline on intestinal control activity.

TABLE 10

The effect of adrenaline on the intestinal control activity (un-notched control potentials)

	No adrenaline	Adrenaline (1×10^{-6}M)
A (mv)	18.0 ± 0.2 (178)	18.9 ± 0.2 (85)*
t_1 (msec)	159.1 ± 4.6 (139)	152.3 ± 5.5 (73)
t_2 (msec)	1000.9 ± 5.9 (139)	1019.7 ± 8.9 (73)
t_3 (msec)	794.5 ± 7.0 (139)	778.6 ± 7.2 (73)
t_4 (msec)	1972.0 ± 12.0 (166)	1959.1 ± 11.8 (85)
t_5 (msec)	1578.8 ± 12.7 (166)	1577.4 ± 8.7 (85)

Values expressed as mean ± standard error of the mean.

Numbers in parentheses refer to the numbers of penetrations

*Statistically significant at the 5% level.

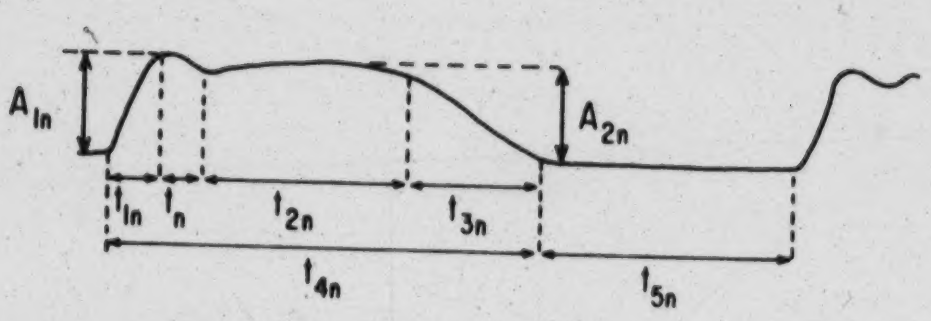

Figure 32: The parameters of the notched control potentials used in the analysis of the effect of adrenaline.

TABLE 11

The effect of adrenaline on the intestinal control activity (notched control potentials)

	No adrenaline	Adrenaline (1×10^{-6}M)
A_{1n} (mv)	17.3 ± 0.3 (32)	18.4 ± 0.4 (24)*
A_{2n} (mV)	16.8 ± 0.3 (32)	18.8 ± 0.4 (24)*
t_{1n} (msec)	140.0 ± 8.4 (32)	139.0 ± 10.6 (21)
t_n (msec)	166.3 ± 8.1 (32)	158.1 ± 9.7 (21)
t_{2n} (msec)	871.3 ± 10.4 (32)	887.6 ± 13.7 (21)
t_{3n} (msec)	785.0 ± 12.9 (32)	763.8 ± 13.2 (21)
t_{4n} (msec)	1960.0 ± 19.0 (32)	1953.3 ± 18.0 (24)
t_{5n} (msec)	1537.5 ± 15.0 (32)	1576.7 ± 14.8 (24)

Values expressed as mean ± standard error of the mean.

Numbers in parentheses refer to the numbers of penetrations.

*Statistically significant at the 5% level.

the maximum 'resting' membrane potential or the control potential amplitude or configuration (Fig. 30). This effect was followed by membrane hyperpolarization of about 6.4 mV. The control potential duration, rates of rise and fall and frequency were not significantly changed by adrenaline even after long times of exposure and at a tenfold higher dose. The hyperpolarization was, however, accompanied by a slight but significant increase in the control potential amplitude. To assess whether adrenaline affected either or both of the initial or secondary components of the control potential, the data were analysed separately for notched (Fig. 31) and un-notched (Fig. 32) control potentials; the results of these analyses are presented in Tables 10 and 11. The intercontrol-potential depolarizations could still be observed in some cells, but since they were normally of variable amplitudes (1-6 mV) and we did not have penetrations in which the electrode remained in one of these cells before and during adrenaline infusions, it is difficult to assess whether their size was changed by adrenaline. The disappearance of response activity was accompanied by cessation, or drastic reduction of the force of, the rhythmic contractile activity and sometimes a decrease in the basal tone of the preparation. Both the electrophysiological and mechanical effects of adrenaline were readily reversible upon adrenaline withdrawal (Fig. 30).

To investigate the possible roles of the membrane permeabilities to potassium and chloride and that of the electrogenic sodium pump in the adrenaline-induced hyperpolarization, we studied the effects of K-free and Cl-free solutions as well as those of temperature on the membrane potential in the absence and presence of adrenaline. This approach was

adopted to overcome the difficulties that arise from the time dependence of the effects of these treatments, particularly the time dependence of the effects of K withdrawal on the membrane potential and the difficulty in keeping an electrode inside the same cell during changing solutions.

The Effect of Adrenaline in K-Free Solutions:

The omission of K in the absence of adrenaline (6 tissues) abolished the intestinal control activity (see Part IV, Chapter 3) and had a biphasic effect on the membrane potential. The membrane initially depolarized from its normal value of -54.82 ± 0.31 to -48.02 ± 0.54 mV in the first twenty minutes. This initial depolarization was followed by a progressive decrease in the membrane potential which finally stabilized at a hyperpolarized level of -60.82 ± 0.49 mV about 30 to 35 min after K withdrawal (Fig. 33). This biphasic effect on the membrane potential is similar to, although of smaller magnitude than, the effects observed in guinea-pig taenia coli by Casteels, Droogman and Hendrickx (1971b) and Tomita and Yamamoto (1971). These authors attributed the initial depolarization to inhibition of the electrogenic Na pump and the subsequent hyperpolarization to an increase in the membrane permeability to potassium. The admission of 1×10^{-6} M adrenaline in the absence of K immediately hyperpolarized the membrane to an average of -59.36 ± to 0.31 mV within the first 20 min after which the membrane potential showed no further decrease (average membrane potential between 30 and 60 min was -61.3 ± 0.33 mV). It is obvious from Fig. 33 that adrenaline hyperpolarized the membrane at the depolarization phase of the response to K withdrawal. If the initial depolarization induced

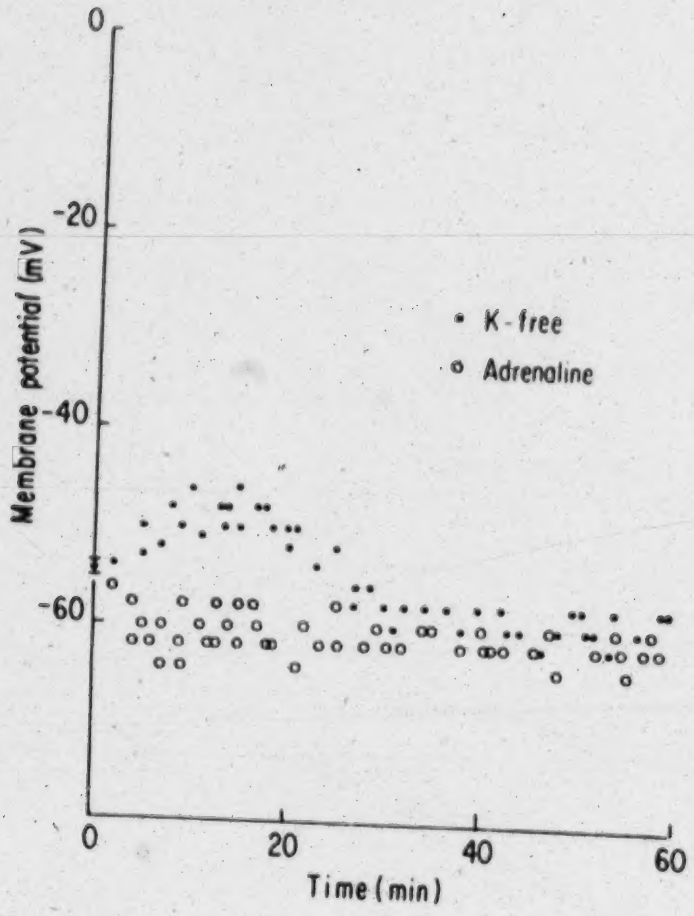

Figure 33: The effect of K withdrawal on the adrenaline-induced hyperpolarization. Dot at zero time represents the average maximum 'resting' membrane potential in normal Tyrode solution at 37° C (bar, standard error of the mean). Solid dots represent membrane potentials at various times in K-free Tyrode solution and open circles represent those in K-free solution containing 1×10^{-6}M adrenaline. Each dot or circle represents one penetration.

by K removal was the result of inhibition of the electrogenic Na pump as suggested by Casteels, Droogmans and Hendrickx (1971b) and Tomita and Yamamoto (1971), then our finding that adrenaline hyperpolarized the membrane at the time when the pump was inhibited by removal of external K argues against the suggestion made by Burnstock (1958) that adrenaline hyperpolarized the smooth muscle cell membrane by stimulating the electrogenic Na pump.

Our finding that, when K withdrawal hyperpolarized the membrane (30 to 60 min after K omission), adrenaline did not cause any appreciable further hyperpolarization may be the result of the dependence of the adrenaline-induced hyperpolarization on the membrane potential. Earlier studies substantiate this explanation; in taenia coli adrenaline hyperpolarized the cells which initially had low membrane potentials and little affected cells the membrane potential of which was initially high (Bülbring and Kuriyama, 1963b).

The Effect of Temperature on Adrenaline-Induced Hyperpolarization:

To further investigate the possible involvement of electrogenic Na pumping in the adrenaline-induced hyperpolarization, we studied the effects of reducing the temperature from 37° C to 16° C (6 tissues). Reducing the temperature to 16° C abolished the electrical control activity of jejunal smooth muscle cells (see Part IV, Chapter 3) and depolarized the cell membranes by about 5.7 mV; the membrane potential stabilized for at least 30 min at about -49.35 ± 0.36 mV (34 penetrations). When the temperature was reduced to 16° C with the simultaneous admission 1 x 10^{-6}M adrenaline, the membrane hyperpolarized from -49.35 ± 0.36 mV to

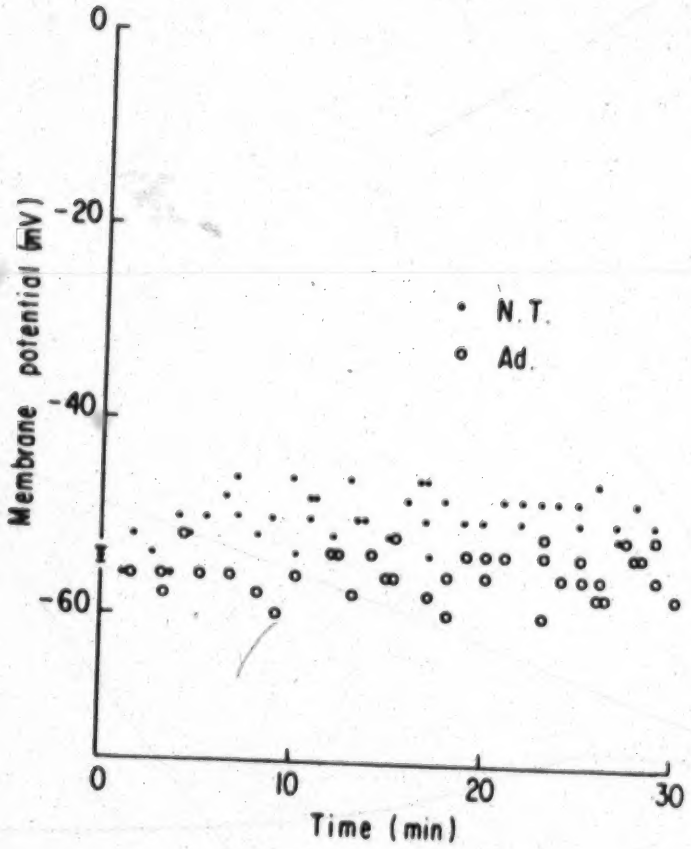

Figure 34. The effect of temperature on the adrenaline-induced hyperpolarization. Dot at zero time represents the average maximum resting membrane potential in normal Tyrode solution at 37° C (bar standard error of the mean). Solid dots represent the membrane potentials of various times after cooling to 16° C and open circles those in Tyrode solution containing 1 x 10^{-6}M adrenaline at 16° C. Each dot or circle represents one penetration.

-55.65 ± 0.39 mV (34 penetrations) as shown in Fig. 34. From these values, it is obvious that adrenaline could hyperpolarize the smooth muscle cell membranes at low temperatures and that the adrenaline-induced hyperpolarization has a low temperature coefficient (Q_{10} = 1.01).

The demonstration of the adrenaline-induced hyperpolarization in tissues in which the Na pump was inhibited by removal of external potassium as well as the insensitivity of this hyperpolarization to cooling provides evidence that the effect of adrenaline on the membrane potential of rabbit jejunal smooth muscle cells is not likely a consequence of stimulation of electrogenic Na pumping. Similar conclusions have been reached from studies on guinea-pig taenia coli (Bülbring and Tomita, 1969b) and rat uterus (Daniel, Paton, Taylor and Hodgson, 1970). Furthermore, our finding that the adrenaline-induced hyperpolarization during the initial phase (depolarization) of K withdrawal effect was larger than that observed in K-containing solution can be easily explained if adrenaline hyperpolarized the membranes by increasing the membrane permeability to potassium ions since reducing the external K concentration would increase the potassium equilibrium potential (E_K).

The Effect of Adrenaline in Chloride-Deficient Solutions:

From the studies in taenia coli some evidence was produced that adrenaline increased the membrane conductance to both potassium and chloride ions. An increase in potassium conductance should bring the membrane potential closer to its equilibrium potential which is about -90 mV in taenia coli (Casteels, 1969; Brading, 1971), that is to say should hyperpolarize the membrane. On the other hand, an increase

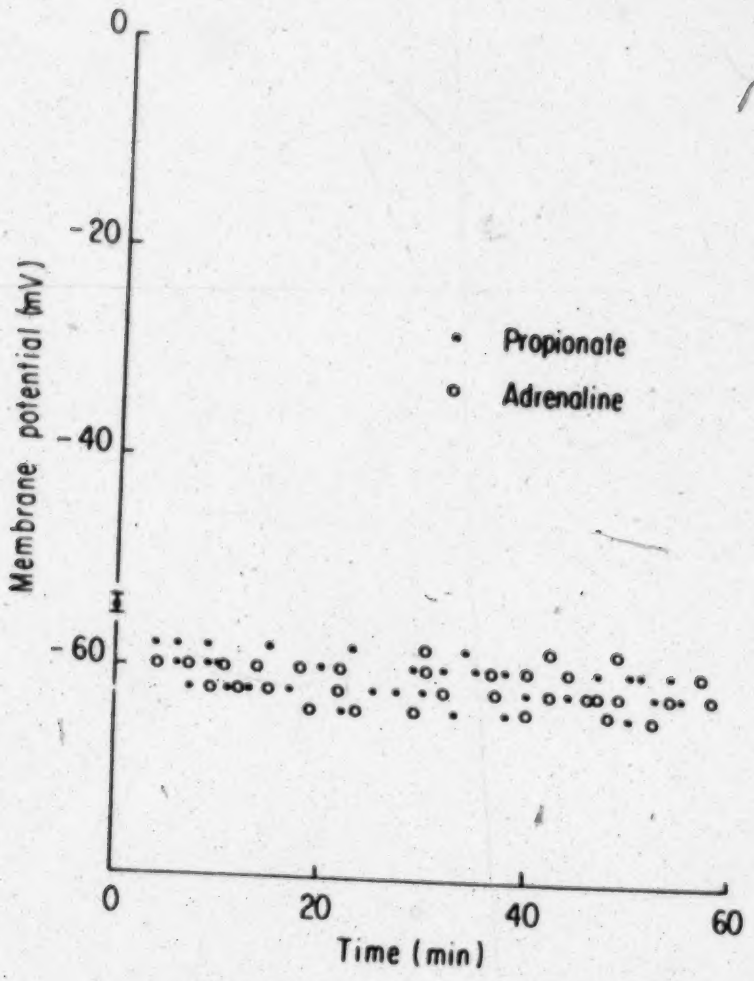

Figure 35: The effect of replacement of chloride by propionate on the adrenaline-induced hyperpolarization. Dot at zero time represents the average maximum 'resting' membrane potential in normal Tyrode solution (bar, standard error). Solid dots represent membrane potentials at various times in propionate-Tyrode solution and open circles represent those in propionate-Tyrode solution containing 1×10^{-6}M adrenaline. Each dot or circle represents one penetration.

in P_{Cl} should shift the RMP towards its equilibrium potential (E_{Cl}) which is about -20 to -30 mV (Casteels, 1969; Brading, 1971), that is to say should depolarize the membrane. If adrenaline had both these effects, then the actual magnitude of the hyperpolarization resulting from the increased P_K should be partially masked by the depolarizing tendency of the increase in P_{Cl}. Thus we decided to study the effects of replacement of Cl^- by the less permeant propionate (5 tissues).

In propionate-Tyrode solution without adrenaline, the membrane hyperpolarized to a new level of -60.82 ± 0.31 (34 penetrations). This hyperpolarization may be due to removing the maximum resting membrane potential away from E_{Cl} and/or to elimination of the chloride current which normally short-circuits the potential generated by the operation of the electrogenic Na pump. Propionate-Tyrode solution drastically decreased the control potential frequency and shortened its duration (these effects were discussed in Part IV, Chapter 3). The admission of propionate-Tyrode solution containing 1×10^{-6}M adrenaline hyperpolarized the membrane to -61.19 ± 0.35 mV indicating that adrenaline did not further hyperpolarize the membrane already hyperpolarized by replacement of chloride by a less permeant anion (Fig. 35). This finding is not consistent with a stimulating effect of adrenaline on the electrogenic Na pump since in the presence of an anion less permeant than chloride one would expect stimulation of the pump to lead to a larger hyperpolarization due to elimination of the shunting effect of the chloride current on the pump generated potential (see for example, Taylor, Paton and Daniel, 1970). It is also difficult to explain if adrenaline increased the membrane permeability to both

K and Cl for in this case one would expect a larger hyperpolarization in propionate-Tyrode solution due to the elimination of the depolarizing effect of the Cl-permeability increase.

DISCUSSION:

The motor inhibitory action of adrenaline on the smooth muscle cells of the small intestine is brought about by the blockade of the response activity (the spontaneous spike discharge associated with the control activity). The cessation of response activity is not the consequence of hyperpolarization but usually preceded it. Nor could it be due to interference with the electrical control activity which continued virtually unaltered in the presence of adrenaline. It is likely that it may be the result of suppression of the prepotentials which precede, and may indeed directly trigger, the spike potentials. In terms of the mechanisms visualized by Daniel (1973) by which drugs, neurotransmitters and gastrointestinal hormones can possibly interfere with intestinal motor function, adrenaline, by suppressing the prepotentials, acts by interfering with the coupling of the myogenic control activity to response activity.

In guinea-pig taenia coli which usually does not show control activity, adrenaline abolished the spike discharge, probably by suppression of pacemaker potentials (Bülbring and Tomita, 1969c) and subsequently hyperpolarized the smooth muscle cell membrane (Bülbring and Kuriyama, 1963b). The hyperpolarization has been attributed to stimulation of an electrogenic Na pump (Burnstock, 1958), to reduction of Na permeability (Bülbring and Kuriyama, 1963b) or to an increase in K permeability partially

TABLE 12

The effect of temperature, potassium withdrawal and replacement of chloride by propionate on the adrenaline-induced hyperpolarization of rabbit jejunal smooth muscle cells

	No adrenaline	1×10^{-6}M adrenaline
Normal Tyrode -37° C	-54.82 ± 0.31 (218)	-61.24 ± 0.38 (113)*
Normal Tyrode 16° C	-49.35 ± 0.36 (34)	-55.65 ± 0.39 (34)*
K-free Tyrode -37° C		
(5-20 min)	-48.92 ± 0.54 (13)	-59.36 ± 0.31 (22)*
(30-60 min)	-60.82 ± 0.49 (17)	-61.63 ± 0.33 (16)
Propionate-Tyrode -37° C	-60.82 ± 0.32 (34)	-61.19 ± 0.34 (27)

Values expressed as mean ± standard error of the mean.

Numbers in parentheses refer to the numbers of penetrations.

*Statistically significant at the 5% level.

masked by a concomitant increase in Cl permeability (Bülbring and Tomita, 1969b; Ohashi, 1971). Evidence presented in this chapter indicates that stimulation of the Na pump, which we have shown to be electrogenic in rabbit jejunal smooth muscle cells (Part IV, Chapter 2), does not appear to underly the membrane hyperpolarization. Adrenaline could hyperpolarize the membranes after the pump had been inhibited by either K omission or cooling (16° C) and replacement of Cl by less permeant anions did not increase the magnitude of hyperpolarization caused by adrenaline (Table 12.

The omission of K initially depolarized the membrane for the first twenty minutes after which a gradual decrease in the membrane potential occurred and the membrane stabilized at a value higher than that in fresh tissues. The initial depolarization and subsequent hyperpolarization may be due to inhibition of the electrogenic Na pump and an increase in K permeability, respectively. Our finding that during the initial phase adrenaline hyperpolarized the membrane to a greater extent than in normal solution is consistent with an effect of adrenaline on K permeability. However, the findings that adrenaline did not further hyperpolarize the membrane already hyperpolarized after 30 to 60 min of K withdrawal or by replacement of Cl by propionate are difficult to explain. In taenia coli smooth muscle cells Ohashi (1971) found that the adrenaline-induced hyperpolarization was greatly reduced or did not occur when the membrane was already hyperpolarized by K removal or stubstitution of benzenesulphonate for Cl ions, respectively. He also confirmed the observations of Bülbring and Tomita (1969b) that adrenaline increased the membrane conductance and that this increase

was reduced in low K or low Cl (benzenefulphate) solutions. To reconcile the discrepancy between the effects of adrenaline on the membrane potential and those on membrane resistance, he postulated that adrenaline increased both K and Cl permeabilities and that these two permeabilities were "not affected in an independent manner when either K or Cl concentration were reduced by substitutin Na or sucrose for K and benzenesulphonate for Cl." Since our results on the rabbit jejunal smooth muscle cells are essentially similar to those of Ohashi (1971) in taenia coli, it is conceivable that the conductance change underlying the adrenaline-induced hyperpolarization is dependent on the Cl concentration. Further work is needed to elucidate this point.

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APPENDIX

THEORETICAL ANALYSIS OF THE CONTRIBUTION OF THE ELECTROGENIC Na-PUMP TO THE TRANSMEMBRANE POTENTIAL

ASSUMPTIONS AND DEFINITIONS:

The assumptions made in this treatment are essentially those assumed in the derivation of Goldman's equation 1 (Goldman, 1943; Hodgkin and Katz, 1949); i.e.,

1. that the electric field may be regarded as constant throughout the membrane,
2. that the activity of an ionic species in the immediate vicinity of either side of the membrane is directly proportional to that of the aqueous solution on that side,
3. that the activity coefficient for the same ion is the same on both sides of the membrane,
4. that the membrane is homogeneous,
5. that in steady state conditions there is no net current crossing the membrane; i.e., the total membrane current $I_m = 0$.

Goldman's assumption regarding the forces governing ion movements is modified in this treatment so as to indicate that two categories of ionic currents cross the membrane (a) passive ionic currents which result from the movements of ions across the membrane under the influence of diffusion and the electric field in a manner which is essentially similar to that in free solution and (b) active

ionic currents that result from the operation of metabolically driven 'pumps' against the electrochemical gradients.

$$E = \frac{RT}{F} \ln \frac{P_K[K]_i + P_{Na}[Na]_i + PCl[Cl]_o}{P_K[K]_o + P_{Na}[Na] + PCl[Cl]_o} \quad \ldots\ldots 1$$

It is further assumed here that chloride movements are either passive or an inward active chloride pump operates and that this pump is electroneutral transporting a positive ion X^+ along with each chloride ion transported. Alternatively the chloride pump may be electroneutral by virtue of transporting another negative ion in the opposite direction. There is reasonable evidence suggesting the existence of chloride pumps in giant axons of the squid (Keynes, 1963) and crayfish (Strickholm and Wallin, 1965; Wallin, 1966), cardiac muscle fibres (Lamb, 1961; Verdonck, De Clercq and Carmeliet, 1965), smooth muscle cells (Casteels, 1967, 1970, 1971), the gastric mucosa (Stevens, 1964; Harrison, Keynes and Zurich, 1968), the intestinal brush border (Frizzell, Nellans, Rose Markscheid-Kaspi and Schultz, 1973) and in toad bladders (Davies, Martin and Sharp, 1968). In nerve and muscle nothing is known about the characteristics of this pump, particularly about its electrogenicity. In other tissues suggestions have been made that it may function in an electrogenic manner, as in gastric mucosa (Hogben, 1955) and in toad bladder (Davies, Martin and Sharp, 1968), or that it may operate in an electrically neutral manner by exchanging chloride for bicarbonate (Dietz, Kirschner and Porter, 1967). In the absence of such information for nerve or muscle, we assumed that the operation of this pump does not provide a significant contribution to the membrane potential.

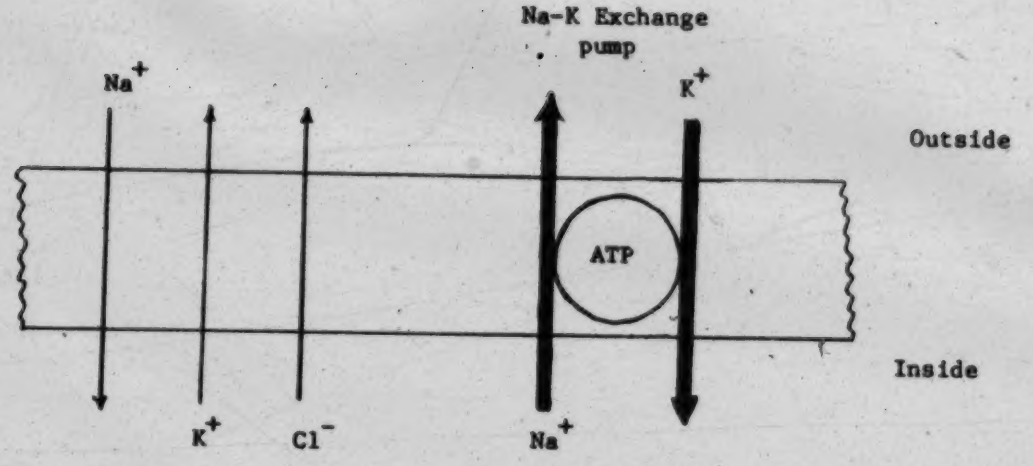

Figure 36: Diagram representing the passive (thin lines) and active (wide heavy lines) ionic movements across the cell membrane. The direction of arrows represent ion flows. Chloride pump not illustrated.

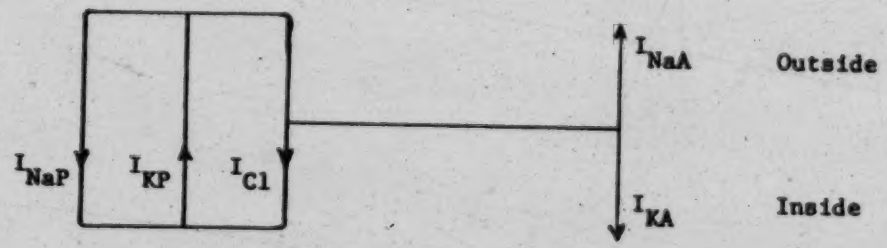

Figure 37: Equivalent circuit for the membrane currents. The direction of arrows represent the flow of positive charges. I the flow of positive charges is inward then the current is given a positive sign in the mathematical treatment.

The model based on these assumptions is diagrammatically illustrated in Fig. 36. Figure 37 shows the equivalent circuit representing the transmembrane currents predicted from the model.

LIST OF SYMBOLS:

E	transmembrane potential
F	Faraday number
J	an ion species
I_J	total (net) current carried by J ions
I_{JA}	the active ionic current carried by J ions as a result of the activity of the Na-K exchange pump
I_{JP}	the passive ionic current carried by J ions under the influence of the electrochemical gradient of J
M_J	net flow of J ions across the membrane
P_J	membrane permeability for J ions
R	gas constant
T	absolute temperature
Z_J	the valency of ion J
$(J)_i$	chemical activity of J ions on the inside of the membrane
$(J)_o$	chemical activity of J ions on the outside of the membrane
$[J]_i$	molar concentration of J ions on the inside of the membrane
$[J]_o$	molar concentration of J ions on the outside of the membrane

α the relative activity coefficient of the Na pump defined as the ratio of active Na efflux to passive Na influx

r the coupling ration of the Na pump, i.e., the ratio of active Na efflux to active K influx.

THEORY:

For any ion J, the membrane current I_J resulting from the movement of J according to the electrochemical gradient is given by

$$I_J = M_J Z_J F \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 2$$

where

$$M_J = - P_J \frac{EZ_J F}{RT} \quad \frac{(J)_o - (J)_i \exp (EZ_J F/RT)}{1 - \exp (EZ_J F/RT)}$$

Substituting the molar concentrations of J for its activities on both sides of the membrane (assumption 3) we get

$$M_J = - P_J \frac{EZ_J F}{RT} \quad \frac{[J]_o - [J]_i \exp (EZ_J F/RT)}{1 - \exp (EZ_J F/RT)} \qquad \ldots\ldots 3$$

From equations 2 and 3 we get the following expression for I_J for monovalent ions.

$$I_J = - P_J \frac{E^2 F^2}{RT} \quad \frac{[J]_o - [J]_i \exp (EZ_J F/RT)}{1 - \exp (EZ_J F/RT)} \qquad \ldots\ldots 4$$

Applying equation 4 for potassium, sodium and chloride we get

$$I_{KP} = - P_K \frac{E^2 F^2}{RT} \quad \frac{[K]_o - [K]_i \exp (EF/RT)}{1 - \exp (EF/RT)} \qquad \ldots\ldots 5$$

$$I_{NaP} = - P_{Na} \frac{EF^2}{RT} \frac{[Na]_o - [Na]_i \exp (EF/RT)}{1 - \exp (EF/RT)} \quad \ldots\ldots 6$$

$$I_{ClP} = - P_{Cl} \frac{EF^2}{RT} \frac{[Cl]_o - [Cl]_i \exp (-EF/RT)}{1 - \exp (-EF/RT)}$$

Multiplying both the nominator and denominator of the last term in the right hand side of this equation by (-exp EF/RT) yields

$$I_{ClP} = - P_{Cl} \frac{EF^2}{RT} \frac{[Cl]_i - [Cl]_o \exp (EF/RT)}{1 - \exp (EF/RT)} \quad \ldots\ldots 7$$

As in Goldman's treatment E is defined as the potential difference across the membrane in the absence of net ionic current; i.e., $I_m = 0$. Hence,

$$I_{Na} + I_K + I_{Cl} = 0. \quad \ldots\ldots 8$$

In a tissue where a sodium-potassium exchange pump operates (Fig. 37), the net ionic currents are given by

$$I_{Na} = I_{NaP} + I_{NaA} \quad \ldots\ldots 9$$

$$I_K = I_{KP} + I_{KA} \quad \ldots\ldots 10$$

From equations 8-10 we obtain

$$I_{NaP} + I_{NaA} + I_{KP} + I_{KA} + I_{Cl} = 0 \quad \ldots\ldots 11$$

To obtain quantitative relationships between passive and active sodium and potassium currents let us define a <u>relative activity coefficient</u> for the pump α as the ratio between the rate of active

sodium efflux to that of passive sodium influx and a coupling ratio r relating the rate of active sodium efflux to that of active potassium influx; i.e.,

$$\alpha = -\frac{M_{NaA}}{M_{NaP}} \qquad \text{.....12}$$

and

$$r = -\frac{M_{NaA}}{M_{KA}} \qquad \text{.....13}$$

From equations 2, 12 and 13, we obtain

$$\alpha = -\frac{I_{NaA}}{I_{NaP}} \quad \text{or} \quad I_{NaA} = -\alpha I_{NaP} \qquad \text{.....14}$$

and

$$r = -\frac{I_{NaA}}{I_{Ka}} \quad \text{or} \quad I_{Ka} = -\frac{I_{NaA}}{r} \qquad \text{.....15}$$

From equations 14 and 15

$$I_{Ka} = \frac{\alpha I_{NaP}}{r} \qquad \text{.....16}$$

From equations 11, 14 and 16

$$I_{NaP} - \alpha I_{NaP} + I_{KP} + \frac{\alpha}{r} I_{NaP} + I_{ClP} = 0$$

or

$$I_{NaP}(1 - \alpha + \frac{\alpha}{r}) + I_{KP} + I_{ClP} = 0 \qquad \text{.....17}$$

From equations 5-7 and 17, we obtain

$$- P_K \frac{EF^2}{RT} \frac{[K]_o - [K]_i \exp(EF/RT)}{1 - \exp(EF/RT)}$$

$$+ (1 - \alpha + \frac{\alpha}{r}) \left(- P_{Na} \frac{EF^2}{RT} \frac{[Na]_o - [Na]_i \exp(EF/RT)}{1 - \exp(EF/RT)}\right)$$

$$+ \left(-P_{Cl} \frac{EF^2}{RT} \frac{[Cl]_i - [Cl]_o \exp(EF/RT)}{1 - \exp(EF/RT)}\right) = 0$$

or $P_K \{[K]_o - [K]_i \exp(EF/RT)$

$$+ (1 - \alpha + \frac{\alpha}{r}) P_{Na} \{[Na]_o - [Na]_i \exp(EF/RT)\}$$

$$+ P_{Cl} \{[Cl]_i - [Cl]_o \exp(EF/RT)\} = 0$$

Expanding and rearranging, we get

$$P_K [K]_i \exp(EF/RT)$$

$$+ (1 - \alpha + \frac{\alpha}{r}) P_{Na} [Na]_i \exp(EF/RT) + P_{Cl} [Cl]_o \exp(EF/RT)$$

$$= P_K [K]_o + (1 - \alpha + \frac{\alpha}{r}) P_{Na} [Na]_o + P_{Cl} [Cl]_i$$

or

$$\exp(EF/RT) \{P_K [K]_i + (1 - \alpha + \frac{\alpha}{r}) P_{Na} [Na]_i + P_{Cl} [Cl]_o\}$$

$$= P_K [K]_o + (1 - \alpha + \frac{\alpha}{r}) P_{Na} [Na]_o + P_{Cl} [Cl]_i$$

$$\exp(EF/RT) = \frac{P_K [K]_o + (1 - \alpha + \frac{\alpha}{r}) P_{Na} [Na]_o + P_{Cl} [Cl]_i}{P_K [K]_i + (1 - \alpha + \frac{\alpha}{r}) P_{Na} [Na]_i + P_{Cl} [Cl]_o}$$

$$E = \frac{RT}{F} \ln \frac{P_K [K]_o + (1 - \alpha + \frac{\alpha}{r}) P_{Na} [Na]_o + P_{Cl} [Cl]_i}{P_K [K]_i + (1 - \alpha + \frac{\alpha}{r}) P_{Na} [Na]_i + P_{Cl} [Cl]_o}$$